Some Experimental Studies on the Erythropoietic Effect of Yellow Bone Marrow Extracts and Batyl Alcohol

BY

OLOF E. SANDLER

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FROM THE HISTOLOGICAL DEPARTMENT
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OF THE UNIVERSITY OF UPPSALA, SWEDEN

Some Experimental Studies on the Erythropoietic Effect of Yellow Bone Marrow Extracts and Batyl Alcohol

INAUGURAL DISSERTATION

By

OLOF E. SANDLER
MED. LIC., NORRL.

By due permission of the most experienced Medical Faculty of the University of Uppsala, to be publicly discussed in the institution of Histology, on March 19th, 1949, at 10:00 A.M., for the degree of doctor of medicine

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To My Family

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PREFACE

Still a student of the clinical side of blood disorders I happened to interest myself in the possibility of treating them with substances derived from the body and thereby increasing the number of blood cells. Clearly, such substances must exist and I set out from the hypothesis that it would be most profitable to start with the blood forming organ itself, i. e. the red bone marrow.

A preparatory study of literature showed, that here and there and particularly in U. S. A. a preparation produced from the nonsaponifiable fraction of yellow bone marrow fat from cattle had been used in agranulocytosis therapy. However, the clinical experiences gained from these experiments were difficult to interpret and therefore I decided to take up the trail, but I made up my mind to employ animal experiments in my investigation.

In this position I turned to the head of the Department of Histology at the University of Uppsala, Professor Martin Wrete, who liberally placed at my disposal all the resources of his Institution. I now take this opportunity of tendering him my sincere thanks for the six years that have passed since then. Without his never failing interest in my research, encouragement and valuable criticism most of the work of which part is presented in this book would probably have remained undone.

The large quantities of bone marrow extracts that have been used have required hundreds of kilogrammes of raw material and this has rendered necessary industrial resources. AB Pharmacia at Stockholm has without expense put all the preparations at my disposal. For this and their friendly cooperation I express my sincere thanks. In this connection I particularly wish to thank AB Pharmacias chief chemist Mr. Erik Askelöf, and the deceased Dr. Phil. Harry Willstaedt for all the trouble and pains they have taken on my behalf.

My sincere gratitude is due the associate Professor of Uppsala University, Einar Stenhagen, and his wife, Mrs. Stina Stenhagen B.Med., for their painstaking work in synthesizing batyl alcohol.

The statistical treatment of my material has been carried out by Gösta Ahlberg F.L. and the actuary at the State Institute for Human Genetics and Race Biology, Mr. Elvir Lander. My heartfelt thanks goes forth to these gentlemen.

For careful and with much interest pursued assistant work I am indebted to my wife Ulla Sandler, my brother in law Holger Kjell, Doctor Ilmar Juhans, Torsten

Hallberg B.Med., Mrs. Sigrid Schybergson and Mrs. Siri Eriksson and to all those who in some way or other have been in touch with this work.

The previous *preparator* at the Institution, Mr. A. Halvardsson and his successor Mr. Tore Wahlund I also thank gratefully for their valuable assistance.

Financial aid has been gratefully received from the Regnell Fund of the Medical Faculty, Svenska sällskapet för medicinsk forskning, The State Funds for the Promotion of Medical Research.

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Uppsala, February 1949.

Olof E. Sandler

Chapter I

INTRODUCTION AND REVIEW OF LITERATURE

A. Introduction

The fact that in internal medicine the science of the blood and its diseases has been given a special name, *hematology*, does not entail that all blood disorders can be looked upon as being specific diseases attacking selectively the blood and blood forming organs only. Cytology having left the purely morphological stage and now also using physical, chemical and biochemical methods, the essential blood diseases gradually decrease in number since the blood disorder as a rule turns out to be only one manifestation of a more extensive damage to a whole organic system or even the entire organism itself. Conversely, this point of view implies that the blood in its capacity of an accessible organ sometimes can be used as an indicator in experimental studies of greater biological importance. A beautiful example of this is the disease sideropenia in which the so called iron deficiency anemia is merely one of the symptoms. The same thing applies to some avitaminous conditions.

Therefore, if a sideropenic with anemia is given iron and the erythrocytes then increase in number it is incorrect to say that the iron is a specific erythropoietic stimulant, i. e. a stimulant affecting the erythrocytes only. The adequate erythropoietic stimulant is an increased demand for erythrocytes, and the concrete expression for this demand is a decreased O_2 tension in the tissues.

Naturally, that which characterizes a living cell is its molecular organization. Hence the molecules foreign to the natural life of the cell can conceivably start a chain of reactions resulting in an increased activity. Consequently no specific stimulation has occurred. This is why so many substances have been discovered with e. g. hemopoietic action, and perhaps more will be discovered. It also explains why clinical applications of laboratory findings often are disappointing; the diseased cells in the clinic are by no means always the same kind of cells as those which in laboratories have been experimentally damaged to reproduce clinical conditions.

Other means also of influencing the erythropoiesis exist in addition to compensating by treatment a state of simple deficiency. Definite, more or less pronounced, changes in the number and quality of the blood corpuscles can, for instance, be demonstrated during the course of some diseases with general toxic effects. In the course of a rheumatic infection, for example, anemia and iron deficiency may be seen simultaneously, and this anemia does not react to administered iron. Not even

an increase in the reticulocytes can be observed. Here we can by using a common expression say that some toxic principle has inhibited the activity of the bone marrow. In respect of the bone marrow a remission can in this case take place in two ways. Either the rheumatic infection abates whereby the production of toxins ceases, or the treatment prevents the toxin from reaching the bone marrow cell, i. e. its permeability to the toxin in question can be changed.

A similar reasoning may be applied to malignant granulocytopenia (agranulocytosis). Numerous attempts have been made to produce so called leukopoietic agents for the treatment of this disorder. However, in this condition it can probably be assumed that the bone marrow is sufficiently stimulated already by the manifested need for leukocytes. The administration of additional bone marrow stimulant is unnecessary, nor does it have the desired result since the bone marrow organ owing to toxic influence cannot respond adequately to an adequate stimulation. The only proper treatment would probably be to somehow induce a detoxication of the diseased organism whereupon the leukopoiesis may start spontaneously as soon as conditions have normalized.

In other words, there is every reason to very restrictively assess all reports of discoveries of so called erythropoietic or leukopoietic principles. We have a typical example in the so called yellow bone marrow concentrate which is the nonsaponifiable fraction of yellow bone marrow fat. As will be seen in the following this preparation has on occasion been used in the treatment of agranulocytosis. In the course of treatment the leukocyte number was often increased and afterwards it even decreased slightly. This increase with subsequent decrease has been interpreted as suggestive of the presence of a leukopoietic principle, the preparation therefore being tried in a series of different conditions characterized by leukopenia and mostly with negative results. The value of the preparation is therefore looked upon with scepsis.

By all this the author does not contend that yellow bone marrow concentrate has no value in the treatment of agranulocytosis. On the contrary, several things indicate that it may be a useful medication and it must not be forgotten that this preparation just like many others may have a defined range of indications, especially as agranulocytosis is not a specific disease but merely a morphologic symptom of chemical or physical attacks on the bone marrow cells. This implies that causal treatment should be varied in accordance with the etiology.

Certain observations can support the assumption that yellow bone marrow concentrate may possess an antitoxic action and that therefore it is under cover of this that the hematopoiesis has time and occasion to get going. Typically, the improvement in the general condition comes very rapidly -- after 1 or 2 days — in the cases "responding" to the treatment, and already *before* any significant improvement in the blood status can be observed. This suggests that a general intoxication is by way of subsiding. The immediately resultant fall in temperature confirms the correctness of the assumption.

The author has treated a number of agranulocytoses with a yellow bone marrow concentrate produced according to C. M. Marberg. However, the material collected is too small to permit definite conclusions to be drawn but sufficient to impulse much reflexion. For, it was striking to see how rapidly and, above all, uniformly most of the cases improved. During the regenerative phase not only the leukocytes returned but also other types of blood corpuscles increased in number, indicating a systematic response by the bone marrow. This the author interpreted as a sign that the preparation had had a more antitoxic and less specifically leuko or erythropoietic action. Hence the effect on the bone marrow was mainly indirect. Naturally, however, there could not be excluded direct stimulation nor a combination of stimulation and protection.

As is easily realized it is a difficult undertaking to formulate a definite opinion of these matters by clinical means. Hence the author decided to attack the problem experimentally in accordance with ramifications laid down in the chapter on "The Problem". First, however, the author desires to review the comparatively few, scattered and in part very hard-to-get publications on the subject.

B. Review of Literature

To obtain clinically useful substances promoting the formation of blood corpuscles by chemical extraction from yellow bone marrow fat is by no means a new idea.

However, all efforts have been centred on the production of substances stimulating the formation of white blood corpuscles for the therapy of cases with agranulocytosis or, generally, conditions of disordered leukopoiesis.

The difficulty, if not the impossibility, of inducing a condition in animals which is identical to agranulocytosis in man is probably the main reason for the paucity of published animal investigations on this subject. Quite naturally, therefore, the majority of publications deal with clinical observations.

Agranulocytosis is in fact a comparatively rare condition which in addition runs a violent course and has a high mortality. This explains why the treatment series published up to the present are far too few and inconsistently dealt with. Save for bone marrow extracts several other preparations have as a rule been resorted to which conceivably might have a stimulating effect on the yielding red bone marrow. If to all this is added the fact that sometimes agranulocytosis heals without any treatment whatsoever the conclusion is unavoidable that small treatment series although at first sight the results seem favourable do not permit of making any definite statement for or against a favourable effect of the used bone marrow extract. In dealing with a disease with about 80 % mortality it is very possible to treat say 10 successive cases with good results without therefore with certainty being able to attribute any sure effect to the adopted therapy.

Since in the present work the author does not in detail deal with leukopoietic

problems works of this nature are of no interest other than in so far as bone marrow extracts have been used. Therefore this review of literature will not include other publications than those in which are discussed the development of the method of producing yellow bone marrow extracts and the few papers on animal experiments.

The author has been unable to find any publications on clinical or experimental investigations into the behaviour of the red blood corpuscles under treatment with yellow bone marrow extracts. Nor has anybody investigated the effect of fractions of the extract and regarding batyl alcohol, which will be discussed in the following, nothing has been published which connects it with erythropoietic questions. In that respect batyl alcohol is in hematology a hitherto unknown substance.

Yellow bone marrow was first reported as a therapeutic agent by GIFFIN and WATKINS (1930). They treated 98 cases of "semiaplastic anemia" and anemia of pregnancy with a fatty suspension of ground and finely strained cattle bone marrow and the attempt met with little success. However, the patients treated also included a case of agranulocytosis which in their view responded remarkably to bone marrows. This finding encouraged the authors to continue and in 1938 they report that since 1930 they had treated 24 cases of agranulocytosis with yellow bone marrow. Some of their patients had had several attacks of the disease which had been treated with yellow bone marrow and cured, except in 3 cases where for some reason or other bone marrow was not administered. JACKSON and TIGHE found the results conducive to continued experimentation but unfortunately not allowing of any definite conclusions because GIFFIN and WATKINS had given far too few details. The same applies to a series described by FLIPSE (1934).

FLIPSE was, however, of the opinion that the bone marrow preparation used up to then was unsuitable since it had to be given in such massive doses that many patients contracted severe digestive disorders. He therefore went to Dr. FENGER in Armour & Co. (Chicago) and ordered a bone marrow concentrate. FENGER in his turn submitted the problem to Dr. MARBERG who evidently is the first to have produced an extract of the nonsaponifiable fraction of yellow bone marrow fat. The production method used is on the whole the same as that described on p. 15 in this work and it accordingly needs not be discussed here.

MARBERG (1938) together with WILES published 6 cases of malignant neutropenia and 4 cases of neutropenia which all recovered after treatment with bone marrow extract. They also mentioned 3 cases of aplastic anemia which were treated without success.

The first experimental work was published by ZICHIS (1936) who by Dennis' method (intraperitoneal insertion of a capsule with 10 ml of a twenty-four hour broth culture of Staphylococcus aureus) tried to induce a leukopenic state in rabbits. These animals were then treated with bone marrow extract. ZICHIS considered that the appearance of leukocytosis already 4 hours after intramuscular injection of the extract would indicate a leukopoietic effect. Unfortunately he reports results from 9 animals only and his paper therefore has a very limited value.

ZICHIS' preparation was later tried without effect by OESTREICHER in benzene depleted rats.

In several respects ZICHIS' method of extracting the nonsaponiable fraction of yellow bone marrow fat so differs from MARBERG's as to make the extraction incomplete. "Two kilograms of bone marrow were saponified with 6 litres of 3 per cent alcoholic potassium hydroxide at room temperature for twenty-four hours. The saponification was aided by mixing the solution with a mechanical stirrer. At the end of twenty-four hours the solution was filtered through a large fluted filter. The residue consisted chiefly of insoluble bony and fibrous tissues. An equal volume of distilled water was added to the filtrate which separated in two layers. The upper layer containing the nonsaponifiable fraction was saved and the lower soapy discarded. In order to free the concentrate from alkali, it was washed 5 times with 50 c. c. of distilled water and 5 times with 50 c. c. of 40 % alcohol. From the original 2 kg of bone marrow, 100 grams of the concentrate were obtained." The method described above was chiefly intended for the production of a preparation to be administered perorally.

An injection preparation was produced as follows: "Two kilograms of bone marrow were saponified exactly as described above. One litre of distilled water was added to the saponified solution, and after shaking thoroughly, 1 litre portions were extracted with 500 c. c. of petroleum ether (boiling point 40—60° C.). After the ether layer separated out, the underlying soap solution was drawn off. When all the saponifying solution was thus extracted, the petroleum ether solution was resaponified with 3 litres of 3 per cent alcoholic potassium hydroxide at room temperature for twenty-four hours. The soap formed by this procedure was insoluble in the liquid medium and was easily separated by filtration aided by suction. The soap was washed 3 times with 50 c. c. portions of petroleum ether. The addition of 1 litre of distilled water to the filtrate caused the formation of two layers. The upper layer was saved and the lower discarded. One litre portions of the petroleum ether solution were washed 5 times with 100 c. c. of 40 % alcohol. When all of the ether solution was thus washed, it was collected and distilled off under diminished pressure at 30° C. The resulting product had a slight cloudiness, but after filtering through a layer of asbestos it remained clear. 20 grams of the concentrate were obtained from the original 2 kg of bone marrow."

Neither of the two described production methods are particularly well chosen. In producing the peroral preparation ZICHIS did not use sufficient alkali for complete saponification (the saponification number of crude yellow bone marrow is in average 170.1). He obtained 50 g of substance from 1 kg of yellow bone marrow while complete saponification yields at most 3 g. He further separated soap solution and nonsaponifiable material with distilled water in such a manner that the mixture separated out in two layers of which only the upper was subsequently extracted with petroleum ether. In order to include all nonsaponifiable matter the lower layer must of course also be extracted.

Table 1. Leukocyte counts in depleted rabbits before and after treatment. (From Caldwell's paper.)

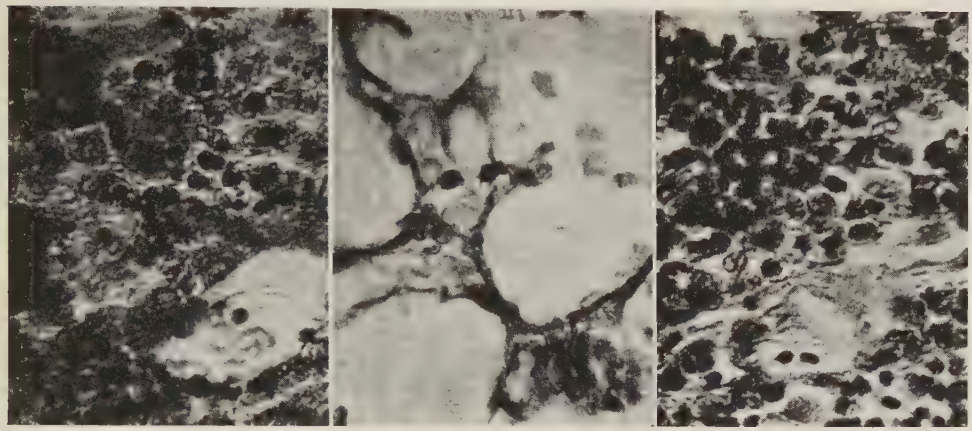
		Group A	Group B	Group C	Group D	Group E
Therapy ($\frac{1}{4}$ cc. daily for 14 days).....		Y.B.M. in olive oil	Olive oil	Cod liver oil	15 units parenteral liver	Carotene concentrate
Number of rabbits depleted		27	27	3	3	6
Total white cells per c.mm. at start of therapy	Mean	*2.331*	*2.044*	*1.933*	*2.133*	*2.516*
	Stand. dev.	477	543	511	603	471
	S.E. mean	±93.6	±106.7	±361	±426	± 208
Total segmented neutrophils at start of therapy (per c.mm.)	Mean	*378*	*249*	*228*	*410*	*278*
	Stand. dev.	492	274	658	154	195
	S.E. mean	±96.6	±53.8	±465	±108.9	±86.2
Number of rabbits surviving therapy...		26	22	3	2	5
Average weight change during therapy .		+192	+20	+220	+57	—10
Condition of bone marrow at end of therapy		Hyperplastic	Aplastic	Aplastic	Aplastic	Aplastic
Total white cells per c.mm. at end of therapy	Mean	*11.403*	*3.376*	*3.017*	*2.550*	*5.240*
	Stand. dev.	3.868	1.933	451	636	1.694
	S.E. mean	±773	±422	±319	±636	±847
Total segmented neutrophils per c.mm. at end of therapy	Mean	*4.473*	*690*	*327*	*192*	*723*
	Stand. dev.	1.863	855	319	153	638
	S.E. mean	±332.6	±186.5	±225	±153	±319

In producing the injection preparation the saponification was slightly more complete which is shown by the fact that here Zichis obtained a yield of only 10 g per kg of raw material, but this was still at least 7 g too much. In both cases the extraction was done with an unsuitable agent, petroleum ether with low boiling point. Petroleum ether with a boiling point of 40—60° C. to a very small extent only dissolves batyl alcohol, for example, at room temperature.

Therefore the clinical and experimental investigations carried out with the substance produced according to Zichis' method have a very limited value.

Holmes et al. have in part analyzed Marberg's bone marrow extract with the object of identifying a factor producing leukocytosis. In so doing they among other things found batyl alcohol but as to its effect on the blood picture nothing was reported, nor did they announce their intention of trying it. These authors were, however, the first to isolate batyl alcohol from yellow bone marrow.

The next experimental investigation was published by the present author (1944) in the form of a preliminary report in Svenska Läkartidningen on the effect of bone marrow extracts on benzene depleted albino rats. A detailed description of this experiment comes later in this work (see p. 24). The investigation demonstrated that the bone marrow extracts counteracted some of the deleterious effects of benzene so that the general condition was improved, the mortality went down and the red blood corpuscles increased in number. A significant difference in the granulocyte counts in favour of the extract treatment could also be observed.



a *b* *c*

Fig. 1. Microphoto of red bone marrow (from Caldwell's paper).

a) Normalplastic control (femur), normal rabbit bone marrow.

b) Aplastic control (femur), benzene depleted, olive oil treated.

c) Hyperplastic control (femur), benzene depleted, yellow, bone marrow treated.

One year later Caldwell, Sifferd, Porsche and Fenger (1945) published a similar experimental work bearing the title "Recent Studies on Yellow Bone Marrow Extracts". They treated 5 groups of benzene depleted rabbits with respectively yellow bone marrow concentrate in olive oil, olive oil, cod liver oil, liver extract (Armour) and carotene concentrate and could in so doing demonstrate a significant increase in the granulocyte count only in the group of animals treated with bone marrow (see table 1, and fig. 1). The therapy lasted for 14 days and in summarizing the authors report" . . . Benzene poisoned rabbits, however, when given comparable doses of yellow bone marrow extracts, showed immediate response and complete *physical*, *leukocytic* and *clinical recovery* by the 14th. day." They drew the conclusion that, "extracts of yellow bone marrow, in contrast to all other materials tested, contain a specific substance or substances, which stimulate leukopoiesis in benzene poisoned rabbits". No significant increase in the erythrocyte counts could be observed which probably must be attributed to the short time of observation.

Chapter II

SUMMARY OF LITERATURE AND THE PROBLEM

It appears from the review of literature that the performed clinical attempts at treating some blood disorders with yellow bone marrow extract are very difficult to judge, there being no theoretical foundation for the method of treatment. To use crude empirism, without regard for ethiological and some topically clinical circumstances such as the degree of development, complication, etc. of the disease, in striving to arrive at a definite opinion as regards the possible value of a method of treatment, may, particularly as regards small experimental series, lead to contradictory results.

In such cases animal experiments are indispensable. The author's animal experiments, to be subsequently described in this work, and those of Caldwell et al. were carried out with preparations which very nearly, respectively altogether, corresponded to the preparation produced by Marberg, demonstrate that benzene depleted animals are beneficially influenced both as regards general condition and blood picture.

Caldwell obtained an increase in the number of granulocytes that he ascribed to the presence in yellow bone marrow of a leukopoietic factor. He found no change in the number of erythrocytes, probably because he observed the animals for a too short period.

The author also obtained an increase in the number of granulocytes but in addition an improvement of the erythrocyte counts. First and foremost I interpreted this result as being dependent on an antitoxic effect on the benzene of the extract.

Just the same a directly stimulating effect on the cells of the bone marrow could not be excluded. In order to find out, the author decided to study animals which could be assumed to be in blood balance, healthy animals in other words. The questions to be answered were as follows:

1) Can parenteral administration of nonsaponifiable fat from yellow bone marrow increase the number of blood corpuscles in animals that show no signs of suffering from blood disease?

2) If such is the case, is it possible to isolate from this fat by fractionation a simple substance with at least the same effect as the unfractionated fat?

3) Is this substance, if any, identifiable?

4) Is it synthetizable?

5) How does the synthetic substance influence healthy man?

Chapter III

THE PRODUCTION OF THE DIFFERENT PREPARATIONS EMPLOYED IN THE EXPERIMENTS

A. Yellow Bone Marrow Preparations

The following exposition of the chemical procedure that was resorted to will only deal with the subject in principle. Figures and unimportant details not required for a true understanding of the subject matter will be omitted as they would unnecessarily make the reading heavy and difficult.

The raw material consisted of yellow bone marrow from cattle. Immediately after the slaughter it was removed from the long tubular bones and refrigerated. While still in a refrigerated condition the marrow was ground to a fine powder.

The ground marrow was saponified with an excess of alcoholic potassium hydroxide solution. The compound was agitated by intromission of nitrogen. After the saponification the compound was diluted with some water and filtered.

The filtered soap solution, which was translucent and dark brown, was repeatedly extracted with ethylene dichloride until the ethylene dichloride layer became practically colourless.

The ethylene dichloride solution was repeatedly shaken with water, whereupon the solution was dried over sodium sulphate and evaporated *in vacuo*. The residue was a semi-solid reddish brown substance which was dissolved in sterile sesame oil mixed with tricresol. The solution was so made up that 1 ml of it corresponded to 40 or 80 g native yellow bone marrow. The yield by this method was 2—3 g unsaponifiable matter per kg of raw material.

The substance thus produced will in this work be called the whole extract or F_0. Now F_0 was tried on benzene-depleted rats (see p. 25). In so doing it was found to be active in so far as it not only had some prophylactic but also a therapeutic effect on the blood picture of the rats. One year later this result was verified by Caldwell, Sifferd, Porsche and Fenger (see p. 13).

The logical consequence of this result was whether it would be possible to isolate a substance or substances possessing an erythropoietic effect. This was in fact accomplished in two stages according to the method described below.

F_0-substance was dissolved in methanol. The solution was chilled to —15° C., a white deposit being precipitated. The precipitate was filtered off and dissolved

in sesame oil so that 1 ml of the solution corresponded to 80 g native yellow bone marrow. The final product was labelled F_3.

The filtrate obtained in the above procedure was chilled to —80° C. and filtered through carbonic acid snow. The new filtrate was evaporated yielding a brownish to brown oil. This oil was used to make preparations as above. The preparation was denoted F_1.

The fraction frozen out at —80° C. was kept, thawed out and evaporated. It constituted a semisolid brownish yellow to brown substance and was the raw material for injection preparation F_2.

The nonsaponifiable fraction (F_0) of the yellow bone marrow had now been broken down into the fractions F_1, F_2 and F_3. The richest yield was obtained in the form of F_2. The four preparations now available were used for a preliminary experiment on otherwise untreated albino rats, the same technique being employed as is described with respect to subsequent experiments. The fraction F_2 exhibited some erythropoietic effect and this substance was therefore further fractionated. In so doing two lines of action were adopted:

I. F_2-substance was completely dissolved in petroleum ether. The solution was then extracted with methanol several times. Thus, the original quantity of F_2-substance was without loss divided into two fractions: the predominantly petroleum ether soluble and the predominantly methanol soluble. The solutions were evaporated to dryness, two liquid semi-solid brownish to brown substances being formed. They were labelled F_2P and F_2M, respectively. Injection preparations were made up in the usual way.[1]

II. The second fractionating method will be described in the form of a detailed example as this is necessary for a correct interpretation of some results obtained subsequently.

26.2 g F_2-substance were dissolved in 275 ml absolute alcohol, with a pipette 27.2 ml glacial acetic acid was introduced into this solution and 3.4 g carbonyl reagent P — according to Girard and Sandulescu — was added to the resultant mixture. The mixture was refluxed on a water bath for 1 hour. Then the reaction mixture was poured into a large separating funnel containing 1 300 ml of water, 16.3 g sodium hydroxide, 550 g crushed ice and 550 ml ethyl ether. The mixture was agitated. When the layers had separated the water phase was shaken twice more, each time with 550 ml ethyl ether. The *ether extracts* were admixed, the fraction F_2A being contained in the result.

To the *water phase* was now added 136 ml concentrated hydrochloric acid and 550 ml ether. The mixture was shaken thoroughly, allowed to stand for 15 minutes, shaken again thoroughly, allowed to stand for another 15 minutes, shaken thoroughly and permitted to separate. The water phase together with 550 ml fresh ether

[1] i. e. all preparations were concentrated to make 1 ml solution equivalent to 80 g native yellow bone marrow.

[2] The method according to A. Girard and G. Sandulesco is described on page 1098 Helvetica Chimica Acta 19—1936.

was subjected to the above procedure and allowed to separate. These two ether extracts were also admixed and their contents labelled F_2K (ketone and aldehyde groups).

The extracts, F_2A and F_2K, were now rid of acid by being washed with a dilute solution of sodium bicarbonate. Then they were dried by filtering through anhydrous sodium sulphate and evaporated. The weight of the residue of F_2K was 2.3 g and of F_2A 24.2 g. Consequently the fractionation was carried out without losses. Both substances were liquid to semi-solid, brownish to brown. As before injection preparations were made up from F_2K.

24.2 g F_2A was now dissolved in 50 ml petroleum ether. The solution was left standing overnight at +2° C. This caused 0.6 g of crystals to be deposited which were purified by many recrystalizations from a methanol solution. The purified crystals were labelled fraction F_2AK and in the usual manner made into an injection preparation.

By evaporation the mother liquor of F_2AK was rid of petroleum ether and yielded 23.6 g of substance from which was produced the preparation F_2A, used in the following.

Summarizing, we consequently obtain the following fractions from yellow bone marrow:

F_0 = nonsaponifiable bone marrow fat.
F_1 = Fraction from F_0, liquid at —80° C.
F_2 = » » F_0, solid at —80° C.
F_3 = » » F_0, solid at —15° C.
F_2M = » » F_2, concentrated in methanol.
F_2P = » » F_2, concentrated in petroleum ether, residue after prep. of F_2M.
F_2K = » » F_2, Made from water soluble fraction, ether soluble.
F_2A = » » F_2, ether soluble, residue of F_2 minus F_2K.
F_2AK = » » F_2A, crystalline.

With red bone marrow, obtained from the breast bones and ribs of calves, as raw material and with the above procedure F_0-substance was produced. This was dissolved in methanol, chilled to —15° C. and analogously $F_1 + F_2$ was obtained. From this the preparation F_2R was produced in the usual manner. The contents of fat in the red bone marrow is naturally less, the yield of F_2-substance therefore being 8 to 9 times as small as when yellow bone marrow was extracted. Since the concentration of the preparation was calculated so as to correspond to 80 g of native red bone marrow, the preparation used became proportionately weaker. The reason for the production of this preparation was a desire to see whether the red bone marrow or associated tissue contained any accumulation of fat-soluble erythropoietic substance. This implies that a smaller dosage was used than for the corresponding preparation from pure yellow bone marrow.

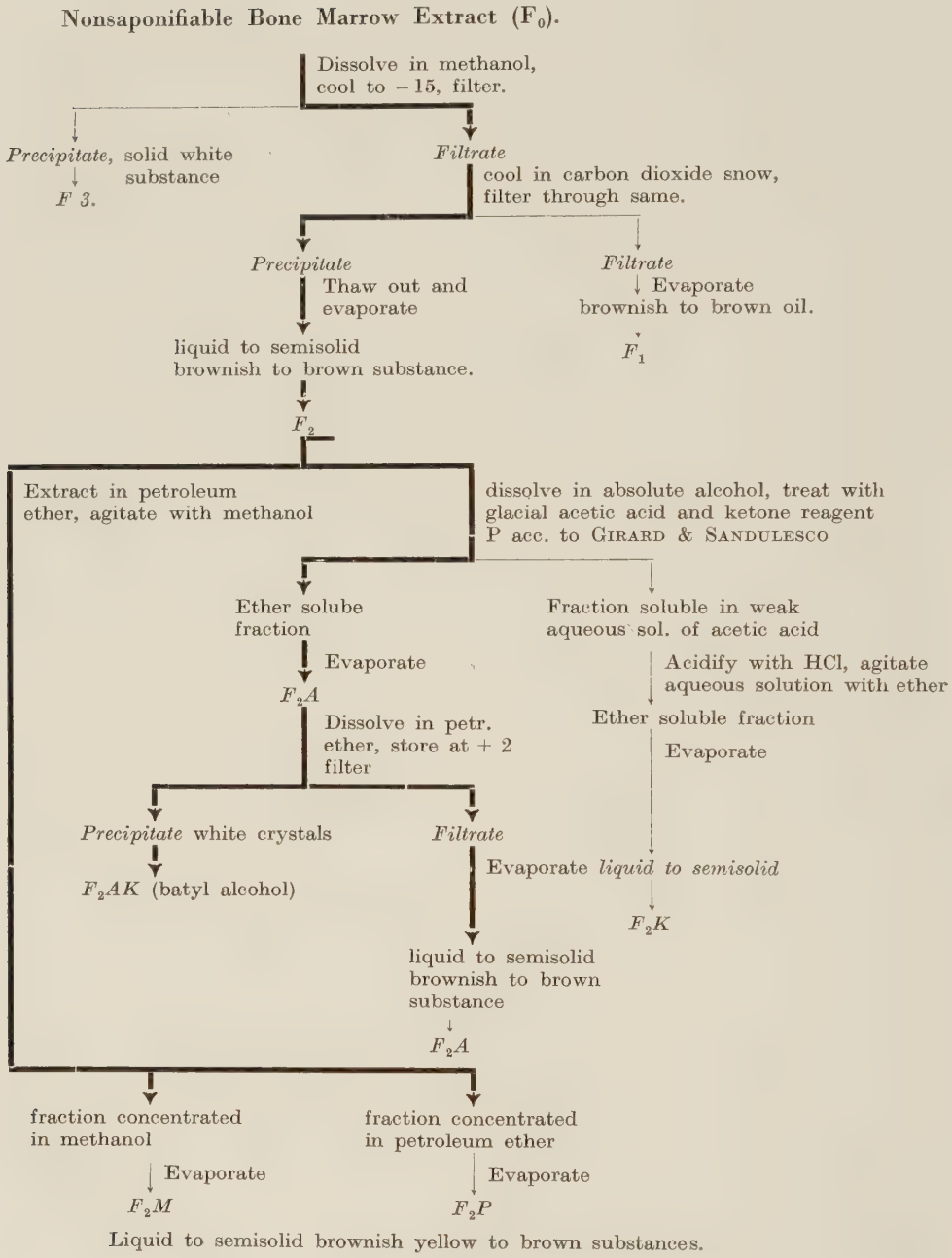


Fig. 2.

Figure 2 illustrates the fractionating procedure from the nonsaponifiable bone marrow extract to the crystalline fraction which is identical to batyl alcohol.

The fractionating procedure is illustrated in outline on p. 18.

A closer study of the F_2AK crystals demonstrated that they were identical to batyl alcohol, which substance will be described in detail in the following. The quantity obtained by the fractionation was small. The final preparation F_2AK contained only 0.3 mg per ml.

It is now possible to divide the preparations into two groups with respect to their batyl alcohol contents. On account of the fractionating method employed it is obvious that F_0, F_2, F_2AK and F_2A must contain more batyl alcohol than F_2K, for example. Batyl alcohol is α-n-octadecyl glyceryl ether and it therefore in the extraction follows the ethyl ether (cf. the production of F_2K and F_2A). Any remnant after the extraction of the water phase must be minute indeed.

In the fractionation of F_2 to F_2M and F_2P the batyl alcohol remains in F_2P, i. e. in the predominantly petroleum ether soluble fraction which is clear from H. N. Holmes' publication 1941. In it he describes the method whereby he isolated batyl alcohol and cholesterin from yellow bone marrow from cattle. He dissolved the non-saponifiable matter in petroleum ether and concentrated the solution in methanol whereupon he obtained batyl alcohol from the petroleum ether phase. This was the first time that the presence of batyl alcohol was demonstrated in other organisms than marine animals.

In which concentration, if any, batyl alcohol is present in the F_2R preparation is as yet unknown.

The sesame oil used in making up the injection preparations wherein the substances were dissolved was according to the standards of the Swedish Pharmacopeia.

Every aseptic safety measure was observed in producing the preparations and from each produced lot a sample was taken which was subjected to bacteriological testing at the Veterinary Institute at Stockholm. However, no bacterial impurities were observed at any time. The final preparation was put into ampoules in a nitrogen atmosphere.

B. Batyl Alcohol

Since batyl alcohol is a substance not found in medico-chemical textbooks the author finds it suitable to give some informative data in connection with the above textual matter. A detailed description is to be found in T. P. Hilditch' book "Chemical constitution of natural fats".

So far three mono-alkyl ethers of glyceryl are known in nature, viz. chimyl, batyl and selachyl alcohols. The chemical structure and the melting points of the three compounds are:

Alcohol	Melting point	Structure
Chimyl	60.5—61.5°	$CH_3(CH_2)_{15} \cdot O \cdot CH_2 \cdot CH(OH) \cdot CH_2OH$
Batyl	70—71°	$CH_3(CH_2)_{17} \cdot O \cdot CH_2 \cdot CH(OH) \cdot CH_2(OH)$
Selachyl	liquid	$CH_3(CH_2)_7CH : CH \cdot (CH_2)_8 \cdot O \cdot CH_2 \cdot CH(OH) \cdot CH_2(OH)$.

While investigating the chemical structure of certain fish oils the presence of these alcohols was first demonstrated in 1922 by TSUJIMOTO and TOYAMA. The latter ascertained that each contained two free hydroxyl groups capable of acetylation. Subsequently ANDRÉ and BLOCH 1932 brought forward evidence to show that in the original fish oils these compounds are present in the form of fatty acid esters, each of the free hydroxyl groups being combined with a higher fatty acid.

From 1928 onwards a detailed investigation into the structure and synthesis of batyl and chimyl alcohols was carried out by HEILBRON and co-workers. They did show that the natural batyl alcohol is an optically active stereoisomeride of the racemic synthetic α-ether with a small but definite optical activity, its specific rotation in chloroform being $(\alpha)^{20}_{5461} + 2.6°$ (C., 0.95).

In 1941 BAER and FISCHER synthesised the optically active form.

At first the presence of these alcohols was demonstrated in certain fish oils, the quantities being greatest in animals belonging to the Elasmobranch group. In 1941, however, batyl alcohol was isolated from yellow bone marrow from cattle by H. N. HOLMES and co-workers, and in 1947 P. KARRER reports that it had been found also in mammalian spleen and aortae.

Evidently batyl alcohol is physiologically present in mammals and probably it also occurs in man but its purpose is not known. In 1947 HILDITCH writes: "The occurrence of these glyceryl ethers in nature is a matter of considerable biological interest. The pure alcohols have neither growth promoting nor antirachitic[1] properties (WEIDEMAN 1924) and their function, if any, in the animal organism is at present obscure."

With the chemical structure in view of the glyceryl ethers they are, as is pointed out by HILDITCH, to some extent an intermediate link between glycerides (true fats) and waxes as shown below.

Glyceride	Glyceryl ether	Wax
$(R \cdot CO \cdot O)_3\, C_3H_5$	$(R \cdot CO \cdot O)_2 \cdot C_3H_5\,(OR')$	$R \cdot CO \cdot O \cdot R'$

Taking batyl alcohol as an example, $R = CH_3\,(CH_2)_{16}$- and $R' = CH_3 \cdot (CH_2)_{17}$-

How these glyceryl ethers are formed in the organism is not known with certainty. LOVERN has pointed out, however, that in the fish oils where these substances occur there always is a subnormal unsaturation in the fatty acids of the oil. The production of the glyceryl ethers could then be interpreted as an expression for an unusual tendency towards saturation or hydrogenation in the fish oils in question.

We shall now compare the formulae for tristearine and the distearine esther of batyl alcohol.

[1] Since it later has been shown that batyl alcohol is physiologically present in the animal body we may be sure that it can be synthezised in the animal. Hence it follows that Weideman's contention by no means must be absolutely true.

$$\begin{array}{l} CH_2O \cdot CO \cdot C_{17}H_{35} \\ | \\ CHO \cdot CO \cdot C_{17}H_{35} \\ | \\ CH_2O \cdot CO \cdot C_{17}H_{35} \end{array}$$

Tristearin

$$\begin{array}{l} CH_2O \cdot CH_2 \cdot C_{17}H_{35} \\ | \\ CHO \cdot CO \cdot C_{17}H_{35} \\ | \\ CH_2O \cdot CO \cdot C_{17}H_{35} \end{array}$$

Distearin esther of batyl alcohol

Here as we see an esther-carbonyl group has been reduced implying hydrogenation of the glyceride molecule. In the liver oil of ratfish all the glycerides have in this manner been reduced to diacyl esthers of selachyl, batyl and chimyl alcohol.

By way of introduction these alcohols were called mono-alkyl ethers of glycerine. Later WEIDEMAN and HEILBRON have demonstrated that batyl alcohol must be a monoglyceryl ether of n-octadecyl alcohol ($CH_3 \cdot (CH_2)_{17} \cdot OH$), chimyl alcohol of n-hexadecyl alcohol ($CH_3(CH_2)_{15} \cdot OH$) and selachyl alcohol of cis Δ 9-n-octadecyl alcohol.

In free form these alcohols have been found in small quantities in various fish oils. Their glyceryl ethers, on the other hand, occur estherized with higher fatty acids.

All the three glyceryl ethers mentioned can be synthesized as has been indicated in the above.

The physiologic importance of these alcohols is, as already has been mentioned, as yet totally unknown. That they possess a pharmacologic action is clear from AGDUHR's investigations into the toxicity of cod liver oil. He showed that in the long run batyl alcohol and octadecyl alcohol may cause degenerative changes in the suprarenal gland, heart, liver and kidneys in albino mice. Another interesting observation is the fact that the free alcohols are far more toxic than their esthers. When AGDUHR gave the animals Greenland shark liver oil, which is very rich in high-molecular alcohols, practically nothing happened. He proved that, in addition, the damage was much more severe when a higher dose was administered. This observation of AGDUHR's may be one reason why the glyceryl ethers occur in nature in the estherized form. Theoretically, therefore, the pure alcohol might very well be assumed to possess an irritating action, which when less intense may constitute a stimulus for certain cells in the organism.

Chapter IV

EXPERIMENTS WITH ALBINO RATS

A. Material and Methods

I. Test Animals

For the investigations presupposed in the chapter on "The Problem" a fairly large number of animals are required. Both for reasons of space and cost albino rats were chosen as test animals. Since different rat strains may exhibit varying normal figures as regards blood cells the problem was to obtain a large number of animals of the same strain. For this reason about 20 animals of the same strain were inbred for rather more than a year. The feeding was strictly standardized. All the time the animals were kept in the same room and as soon as the young left their mother they were from then onwards given a standardized so called rat bread[1], delivered from the Cooperative Bakeries at Stockholm.

Only adult animals were used in the experiments, the reason being that during growth the blood picture of the rat changes typically. In each experimental series a like number of males and females were used, and the sexes were kept apart in the cages to prevent pregnancy.

II. Blood Sampling

For each animal blood specimens were taken at about the same time in the morning and by one and the same person in each experiment to eliminate the personal factor in taking the specimens.

In blood sampling it proved necessary to anestethize the animals and this took place by means of ether narcosis. In doing this the animals were let into a glass jar of 10 litres volume on the bottom of which a layer of ether-moistened sawdust was strewn. Narcosis at an adequate level set in almost immediately. The narcosis was, however, not allowed to become deeper than to permit the animals to wake up

[1] 100 litres separated milk,
47.7 kg spice-free bread crumbs,
21 kg oat groats,
15.75 kg wheat sprouts,
10 kg Luzerne hay flour,
2.5 kg meat meal,
40 g fish liver oil.

immediately after the specimens had been taken. Nevertheless, solitary cases of death in narcosis occurred.

To obtain the blood specimens a small piece of the tail was cut off each time and due care was taken that the incision was made in for macroscopic inspection suitable tissue. In so doing a fairly liberal flow of blood is obtained without need for preliminary heating of the tail or manual expression of blood. Blood sampling having been completed, the surface of the wound was coagulated with a searing iron. The great advantage of this method is that it is the only one permitting repeated sampling. Its disadvantage is that hemorrhages later may occur owing to manipulations by the animal itself or its fellows in the same cage. Infections of the wound in the tail were fairly uncommon.

Each time blood was taken the specimen was intended for determinations of red and white blood corpuscles, two smears for differential counting and a portion for counting reticulocytes. This means that the operator in question had to take up to 300 specimens daily.

III. Counting of the Red Blood Cells

In each experiment one and the same person always counted the red blood corpuscles. The counting was performed with the aid of a binocular microscope and always in the same squares in the same counting chamber.

The specimen for red blood cells was taken in an ordinary Hellige pipette and Hayem's solution was used as a diluent. Since the albino rat by nature is polycytemic blood was only drawn up into the pipette to the 0.5 graduation so that the resultant dilution became 1:200, thus avoiding the necessity for too many blood cells in the counting chamber. In it were counted 8 CD squares and the resulting number was multiplied by 2. Since I as far as possible have endeavoured to systematize errors of method and because in this work it was unnecessary to determine erythrocyte numbers characteristic of a particular situation but merely the *difference* between the erythrocyte numbers in some animal series, no determinations were made of the error of method due to the workers or instruments used. Accidental errors in observations or technique contribute to the individual variation in the animals from one observation to the next, and due regard has been taken hereof as appears from the statistical reasoning in the respective parts of this study.

IV. Injections of the Various Preparations

Once daily the animals were given the injections intraperitoneally and apparently this route of administration did not cause them any trouble, nor could at autopsies, if any, be observed any morbid disorders that conceivably could have been induced by the various bone marrow fractions or the solvent, sesame oil.

B. Experiments with Benzene Depleted Albino Rats

I. Introduction

It is now well known that some time's exposure to benzene will yield grave changes in e. g. the blood picture in both man and animals. Above all these changes are characterized by a progressive reduction in the number of blood corpuscles. As regards the erythrocytes this reduction is brought about in several diverse ways. Benzene acts both directly on mature erythrocytes, resulting in hemolysis, and on their primary evolutionary stages in the bone marrow so that in time aplasia sets in. Indirectly also the benzene reduces the number of blood corpuscles because, owing to endothelial damage, hemorrhages sometimes occur, e. g. from mucous membranes in the digestive tract. Another indirect effect, which must not be disregarded in test animals, is the intensive action of benzene on the central nervous system which in its turn contributes to the deterioration of the general physical condition.

The chronic form of benzene intoxication, i. e. the condition arising from continuous exposure to benzene, is invariably fatal. In principle there is no difference between the clinical and the experimentally induced form of intoxication.

There are available several publications dealing with experimental chronic benzene intoxications, the most recent probably being Selling's (1911). By means of subcutaneous benzene injections he induced a blood-bone marrow disorder in rabbits which finally resulted in total aplasia of the entire hemopoietic system.

II. The Experiments

With the object in view of finding out whether the nonsaponifiable fat in yellow bone marrow could beneficially influence the blood picture in animals suffering from bone marrow disorders an experiment was carried out with benzene-depleted albino rats. The experiment (which originally had a main purpose of studying the white blood picture) has already been published in the form of a preliminary report to "Svenska Läkartidningen" (1944) and will be reproduced below in a slightly amplified form as regards erythrocyte counts, mortality etc.

The period of observation embraced the time from 27.9—27.11 (62 days) and during this interval all the animals were given benzene. The benzene (according to the Swedish Pharmacopeia) was diluted in sterile olive oil to an amount of 30 %.

Initially the benzene was injected subcutaneously but as from the 16th. day it was given intraperitoneally since the first route of administration exhibited a tendency to cause subcutaneous abscesses at the site of the injection. After the alteration in injection procedure this complication did no longer arise, probably owing to a more rapid resorption from the peritoneum.

The benzene dosage varied between 0.2 and 0.4 ml depending on the general physical condition of the animal, implying that the lower dose chiefly was administered to the solely benzene treated animals which exhibited both the worst physical condition and the highest mortality. Briefly, diseased animals were given smaller doses. This safety measure is necessary since otherwise the risk is run that the animal dies in acute benzene intoxication. Very soon after the injections symptoms were manifested from the central nervous system in the form of severe tremor and convulsive seizures lasting from 1 to 2 hours.

The bone marrow preparation used was F_0 (see p. 15) so concentrated that 1 ml corresponded to 40 g of native yellow bone marrow. F_0 was injected in the same manner as the benzene. In the animals given F_0 the dose was varied as follows: 27.9—7.10, 0.1 ml; 8.10—2.11, 0.2 ml; 3.11—6.11, 0.3 ml; 7.11—14.11, 0.2 ml; and from 15.11 to the end of the experiment 0.3 ml per day. No formation of abscesses was at any time recorded following the F_0 injections.

a. Results

Series 1. Of the animal series included in the experiment two are immediately comparable with respect to a study of the behaviour of the erythrocyte count. In one of these series, comprising 40 animals (nos. 9—48), the animals were daily given *benzene injections* only. Within 30 days 13 animals or 32.5 ± 7.4 % in this series had died and *the mean erythrocyte number in the 27 surviving animals was* 5.43 ± 0.19 *mill.*

The second series comprised 16 animals (nos. 49—64) which during the same period were treated with *benzene plus the bone marrow preparation* F_0. In this time 3 animals or 18.8 ± 9.8 % died. *The mean erythrocyte number in the 13 surviving animals was* 6.50 ± 0.37 *mill.* (see diagram 2).

A comparison of these two series, which had been treated concurrently for about a month, makes it clear that the animals which in addition to benzene were given F_0 had a higher erythrocyte number at the end of the period of observation. The difference between the two erythrocyte numbers is 1.07 mill. The test ratio (t) — according to R. A. Fisher — is 3.0 and $P < 0.003$, the difference therefore being considered statistically significant.

The difference in mortality is 13.7 ± 12.2 % and is consequently not significant.

11 (nos. 25—40) of the 27 animals in the series 9—48 surviving after one month of benzene treatment were subjected to the same regimentation for another month. Concurrently the benzene $+F_0$ treatment was continued in the 13 surviving animals in the series 49—64. By this means the result of two months' treatment could be observed (see table 2 and 3 and diagram 1 and 2).

Series 2. In both series for which data has been collected in the above tables the number of animals at the beginning was 16. Up to 25.11 14 solely benzene treated

Table 2. The erythrocyte count after 2 months' benzene depletion of 16 albino rats.

Animal no.	Observation Day							
	29.10	1.11	3.11	7.11	10.11	15.11	18.11	25.11
	Erythrocyte count							
*25	dead							
26	[1]5.30	5.86	5.82	5.64	6.20	5.60	4.54	4.24
27	dead							
28	dead							
29	6.75	6.90	7.00	7.24	6.50	dead		
*30	dead							
*31	5.60	6.46	7.60	dead				
*32	6.02	6.14	6.70	7.12	7.72	dead		
33	3.82	dead						
34	3.30	4.50	4.92	3.68	dead			
35	4.70	5.42	7.08	dead				
36	3.52	dead						
37	6.66	7.62	6.80	6.04	6.66	3.24	dead	
38	5.18	3.88	5.20	6.02	5.88	5.52	5.26	4.14
39	5.20	5.96	6.48	6.06	6.00	2.98	3.12	dead
40	dead							
Mean	5.10	5.86	6.40	5.97	6.49	4.44	4.31	4.19

[1] Figures in mill./c. mm.

* no abscess noticed.

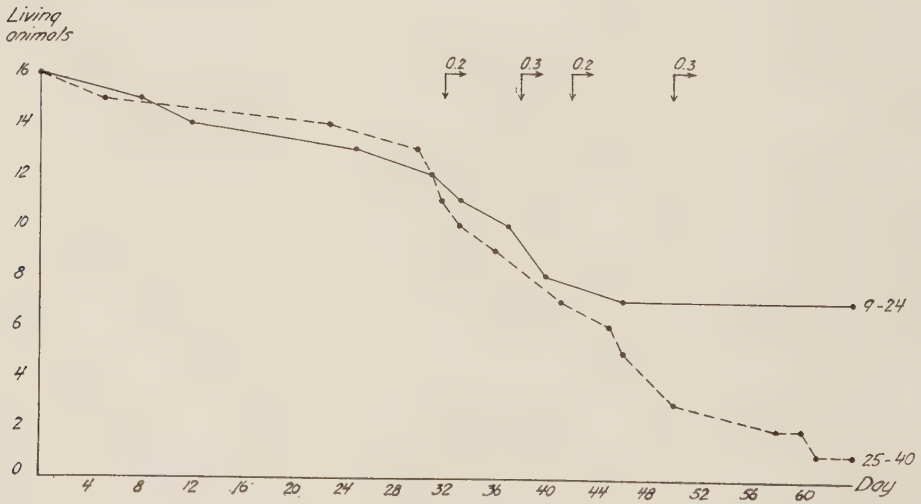


Diagram 1. The continuos line represents the mortality in animals, that first were given benzene only and from the 32nd day benzene + F_0. The broken line (25—40), mortality by animals treated solely with benzene. The arrows indicate initiation of daily doses of F_0 0.2—0.3 ml respectively.

Table 3. The erythrocyte count after 2 months' treatment with benzene + F_0 of 16 albino rats.

Animal no.	Observation Day					
	29.10	2.11	5.11	8.11	17.11	25.11
	Erythrocyte Count					
49	[1]5.86	3.26	3.02	dead		
50	4.16	3.94	dead			
*51	dead					
52	dead					
53	6.08	dead				
54	5.92	dead				
55	6.52	3.52	4.18	5.50	5.58	6.52
56	6.20	7.20	7.34	6.78	7.28	6.18
*57	8.42	[2] ?	6.44	6.30	8.26	6.72
*58	6.48	?	6.24	5.68	7.50	8.98
*59	8.16	?	7.74	8.22	dead	
*60	7.84	dead				
61	4.26	?	6.26	?	6.42	7.80
62	7.42	?	8.40	6.18	7.30	8.58
63	7.24	?	6.72	6.70	7.56	7.26
*64	dead					
Mean	6.50	—	6.26	6.48	7.13	7.43

[1] Figures in mill./c. mm.

[2] no observation.

* no obscess noticed.

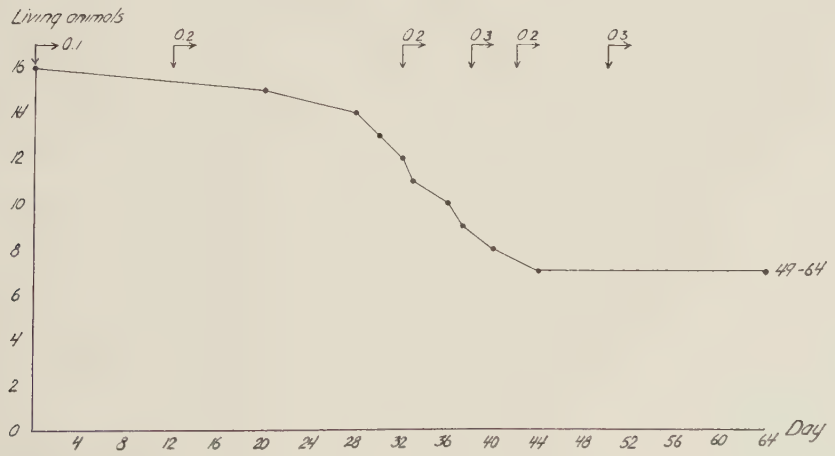


Diagram 2. The mortality in animals, 49—64, from the beginning treated with benzene + F_0. The arrows indicate initiation of daily doses of F_0 0.1—0.2—0.3 ml respectively.

Table 4. Erythrocyte number in animals that up to 29.10 were treated with benzene only and thereafter with benzene + F_0.

Animal no.	Observations Day							
	29.10	1.11	3.11	7.11	10.11	15.11	18.11	25.11
	Erythrocyte Count							
9	[1]6.24	5.38	4.68	6.28	5.04	4.14	4.62	5.48
10	7.32	6.80	4.80	5.30	6.92	6.24	5.60	6.62
11	4.76	5.94	5.40	4.90	6.52	dead		
*12	5.32	5.80	5.96	5.26	5.24	4.82	4.46	5.04
*13	dead							
*14	5.60	5.92	4.74	5.50	5.56	6.70	7.56	7.14
15	4.48	3.30	4.84	dead				
16	6.12	5.74	5.66	6.32	5.58	7.66	6.60	7.26
17	5.36	5.36	6.30	[2] ?	6.74	6.16	7.02	5.72
18	4.90	5.50	7.02	dead				
19	dead							
20	5.70	5.82	6.24	6.30	6.50	6.50	6.52	6.32
21	dead							
22	dead							
23	4.52	3.94	dead					
24	6.04	dead						
Mean	5.53	5.41	5.56	5.69	6.02	6.03	6.05	6.23

[1] Figures in mill./c. mm. [2] no observation. * no abscess noticed.

animals died (table 2) and the erythrocyte count of the 2 surviving animals was 4.24 and 4.14 mill., respectively. The death rate was 87.5 ± 8.3 %.

During the same period 9 animals or 56.3 ± 12.4 % died of those treated with benzene + F_0 (table 3). The mean erythrocyte number for the 7 surviving animals was 7.43 mill. (the lowest figure was 6.18 mill.).

According to Fisher's method the probability that random factors have influenced the difference between the erythrocyte numbers of the surviving animals is 0.005 (the test ratio t = 4.5; degrees of freedom 7). The probability of 0.005 is negligible and the difference must therefore be considered significant.

The difference in death rates between the two series was 31.2 ± 14.9 %.

Consequently observations of these animals after 1 and 2 months, respectively, yielded compatible results.

Series 3. There is a third, additional method of testing the results of the treatment and that is by comparing two more groups of animals. In this case also, owing to the high mortality, the number of animals that can be observed at the end of the experiment is small.

One of these groups comprises 12 animals (nos. 9—24) of those that previously had been treated with benzene only for 1 month and which during the following month were given benzene and F_0 (see table 4).

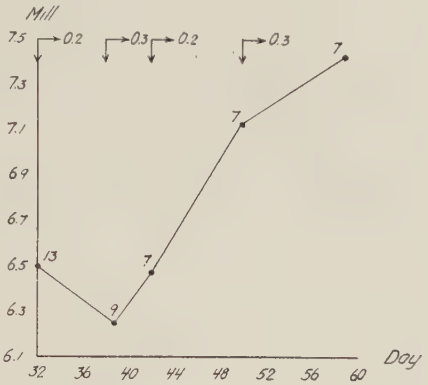


Diagram 3. Mean erythrocyte numbers of animals 49—64, from the beginning treated with benzene + F_0. The arrows indicate initiation of daily doses of F_0 (see diagr. 2 above). The figures in the graph denote the number of surviving animals on the different occasions.

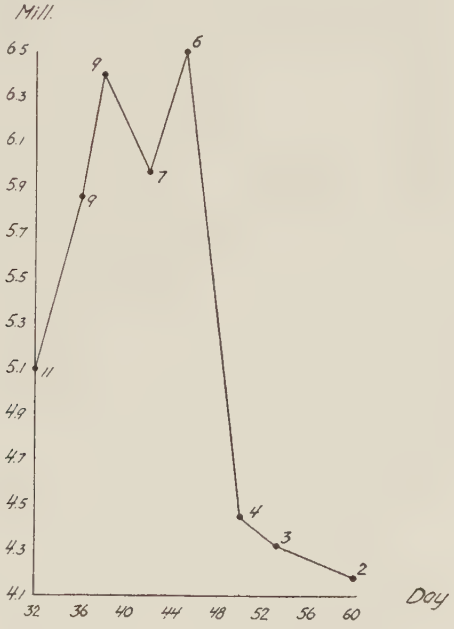


Diagram 4. Mean erythrocyte numbers in animals nos. 25—40 after two months benzene injections. The figures in the graph denote the number of surviving animals on the different occasions.

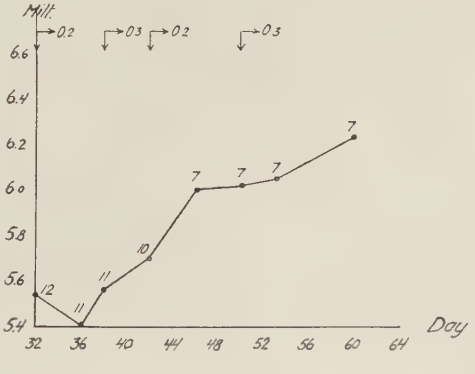


Diagram 5. Mean erythrocyte numbers in animals nos. 9—24, that up to the 32nd day were treated with benzene only and thereafter with F_0 + benzene. The arrows indicate the initiation of daily doses of 0.2—0.3 ml F_0. The figures in the graph denote the number of surviving animals on the different occasions.

During the period of observation 5 animals died in this series or 42 %. The mean erythrocyte number of the 7 surviving animals was 6.23 mill.

The second series (table 2) comprised 11 animals that for about 1 month had been treated with benzene only. In these the treatment was continued for another month and at that time (end of observation period) but 2 animals survived (they also died shortly afterwards). It follows that the death rate was 82 ± 11.6 %.

The difference between the erythrocyte numbers of the two series is 2.04 mill. and by Fisher's method the probability will be 0.015 (test ratio $t = 3.30$) that the difference is not a random one.

The difference in mortality is 40 ± 18 %.

The result of the comparison just made accordingly points in the same direction as do the preceding series, i. e. the bone marrow preparation F_0 has to some extent combated the effects of a chronic benzene intoxication.

The numerical matter in the above exposition is below plotted graphically in diagrams 1—5.

b. Discussion

In addition to the dissimilarities in blood picture and mortality the animals treated with F_0 differed in some other respects from those treated with benzene only.

Benzene, repeatedly injected subcutaneously at approximately the same spot fairly rapidly causes necrosis. This fact was observed already by SELLING. The subcutaneous necrosis soon enough passes into an abscess which, the skin covering having become necrotized, empties. In 3 of my cases (nos. 21, 30 and 52) the abscess instead perforated the abdominal wall inwards resulting in peritonitis. None of these animals were alive when the erythrocyte counts were taken.

Since all the rats in my experiments were treated with benzene for the same duration and in the same manner they should have reacted alike. Actually this was not the case. On the 12.10, i. e. after the 16 days during which the animals were given benzene once daily, not less than 19 of the solely benzene treated animals had abscess while only 3 of those treated with benzene and F_0 exhibited the same disorder. In both series the animals initially were 40 in number. In the benzene series 34 animals survived of which 19 or 55.9 % had visible abscesses, and in the benzene + F_0 series 38 animals of which 3 or 7.9 % had abscess.

As from the 12.10 the author administered all the injections by the intraperitoneal route. In some animals the necrotic processes continued to form around the previous injection sites, the result being that on the 20.10 the distribution was 27 and 15, respectively, which was the maximum.

In tables 2—4 the animals are marked with an "*" that at no time exhibited visible abscesses. Evidently the distribution of animals with abscess in the series here compared is exactly the same. The effect of the abscesses on the condition of the animals is therefore presumably more or less equal. The tables indicate no

certain connection between the abscesses on the one hand and greater changes in the erythrocyte number or the mortality on the other. Nor was there any noticeable effect on the general physical condition or the behaviour while the experiment was in progress other than that the animals treated with F_0 were in much better condition than the others; a circumstance on a later occasion also observed by Caldwell.

In the animals compared above the distribution of benzene necroses and abscesses, respectively, was even, and there was no certain connection between the presence of abscesses and marked changes in the erythrocyte number. (See tables 2—4). Naturally an abscess to some extent reduces the number of erythrocytes because of bone marrow toxic substances resorbed from the necrotic area. (Emphasized on p. 35 of this report.) Most probably, however, this slight reduction can safely be disregarded owing to what already is known as to the anemizing action of benzene. Therefore, in *this* case the author does not consider the presence of abscesses a disturbing factor in estimating erythrocyte numbers or mortality.

However, one circumstance in connection with the abscesses is worth noting. It is described on p. 30 and relates to the difference in frequency and moment of observability in benzene depleted rats with and without F_0-substance. On a given date abscesses observable by external inspection occurred in 55.9 % of the animals treated with benzene only while merely 7.9 % in the other series exhibited the same symptoms. The difference is statistically significant: 48.0 $\pm$ 9.6 %, and it is related to the difference in physical condition which was very obvious to the observer although impossible to exactly express numerically.

The dissimilarities recorded here in the animal series under comparison as regards the erythrocyte counts and the incidence of purely local benzene damage must be taken as being characteristic of the same thing, viz. unequal power to resist the cellulicidal action of benzene. This can only be due to the difference in treatment, i. e. the F_0 preparation did have a beneficial effect.

Numerically speaking the differences in death rate are not statistically significant and cannot be made so with the small experimental series used. The difference is at most approximately 2 times the mean error of the mean. But if we take a look at the death rate curves (diagrams 1—2) we find that the mortality is remarkably consistent in the three series under comparison up to and including the 46th. day of treatment whereafter the mortality tapers off for the animals given F_0 and remains constant for the animals given benzene only. The alteration in the course of the mortality curves coincides temporally with the incipient increase in the erythrocyte counts (cf. diagrams 3—5), it therefore in this case most probably being a characteristic of the efficacy of the treatment.

As regards the importance of alterations in the amounts of F_0 substance administered the beneficial effect apparently sets in only when the F_0 dose is increased to 0.3 ml per day. Caldwell's experiences (p. 13) were essentially the same.

c. Summary

By experimentation on benzene depleted albino rats the author has shown that administration of the nonsaponifiable fraction of the yellow bone marrow from cattle

1) increases the power of the animal to resist the effects of continued exposure to benzene, which was evidenced by an improved general physical condition and lowered mortality.
2) increases the number of erythrocytes in the blood.

C. Experiments with Healthy Albino Rats

These experiments refer to the red blood picture in the albino rat following injections of various yellow bone marrow extracts.

I. Material and Methods

The material comprised 160 adult albino rats — 80 males and 80 females — all being of the same strain and fed a standardized so called "rat bread" (see p. 22).

The injection preparations were all concentrated so as to make 1 ml correspond to the yield, obtained with the extraction method adopted, from 80 g of native yellow or red bone marrow, respectively, from cattle. Using the designations adopted elsewhere, the preparations were F_0, F_2, F_2M, F_2P, F_2A, F_2AK, F_2K and F_2R. Further details are given in Chapter IV which deals with the extraction procedure.

The animals were divided into 8 groups of 20 animals each. Each group contained the same number of males and females. 4 animals of the same sex were kept in one cage.

Thereafter the 8 injection preparations were each given to one group, in the text labelled like the preparation in question, e. g. the F_0-group, F_2-group, etc.

The experiment lasted from 3.7 to 26.8. In order to simplify the work by reducing the number of animals observed daily, they were divided into three groups which successively went into and out of the experiment according to the plan below.

Group	Bloodsampling starts	Injections start	Observation ends
F_0	3.7	17.7	24.8
F_2	»	»	»
F_2M	»	»	»
F_2P	4.7	18.7	25.8
F_2A	»	»	»
F_2AK	»	»	»
F_2K	5.7	19.7	26.8
F_2R	»	»	»

Each phase in the observations was carried out by one and the same person and using the same technique. One took the blood specimens and gave the injections, another counted the erythrocytes, a third the leukocytes, etc.

The blood specimens were always taken in the same rotation so that each observation for the same individual animal came to take place at roughly the same time of day. This reduced to a minimum any variation in the individual blood values due to daily rythm. Naturally the same plan was used in the counting of the specimens so that each pipette with blood was lying waiting an equal length of time before the erythrocyte number was determined.

Each animal was observed every third day, sundays excepted, so that 16 observations were obtained from each animal. The total observation time was divided into 5 periods. During the first two weeks (comprising 5 observations) no injections were given. This period was therefore called the *normal period*. The remaining 11 observations were distributed over periods I, II, III and IV of which the last comprises two observations and the others 3 observations each.

During periods I—II 0.25 ml of the substance in question was injected daily by the intraperitoneal route following blood sampling, and during periods III—IV 0.5 ml.

Further procedural particulars are recorded in Chapter IV.

II. The Progress of the Experiments

During the time of observation some deaths occurred among the animals. These were due both to the experimental conditions as such (ether narcosis, injection damage, etc.) and to diseases which quite independently of the experiment always are liable to occur among albino rats. Bronchopneumonia, for example, is well known to everyone who has had the opportunity to work with albino rats. Sometimes an epidemic intestinal disease may become rampant and severely ravage the stock of animals. Fortunately such was not the case with this group of animals. The author has assorted the reasons for the fatalities in the tables below. Whenever the cause of death could be ascertained it was found that mostly the manipulation of the animals was to blame. When the cause of death is given as "unknown," this implies that no postmortem examination of the body was carried out which, however, must not be taken to mean that in these cases also the narcosis should *not* be the predominant cause of death. As a matter of fact the mortality — 20 animals out of 160 — approximately corresponds to what the author's experience would lead him to expect in this kind of experiment with albino rats.

During the time of observation the animals were on the whole healthy. A report containing any remarkable incidences was made out for each animal. In this connection suppurative inflammations are most interesting as these have been known to negatively influence the formation of erythrocytes when they have become suf-

Table 5. Cause of death in the various animal groups.

F_0		F_2		F_2M		F_2P	
Date	Cause	Date	Cause	Date	Cause	Date	Cause
10.7	Narcosis			6.7	Narcosis	1.8	Bronchopneumonia
27.7	Narcosis			14.7	Unknown	2.8	Unknown
				1.8	Unknown	9.8	Unknown
27.7	Faulty injection			14.8	Broncho-pneumonia	11.8	Narcosis
27.8	Narcosis					20.8	Unknown
						22.8	Narcosis
Total deaths	4		0		4		6

F_2A		F_2AK		F_2K		F_2R	
Date	Cause	Date	Cause	Date	Cause	Date	Cause
28.7	Narcosis	29.7	Unknown	9.8	Peritonitis (puncture)		
				9.8	Narcosis		
				9.8	Faulty injection		
				19.8	Narcosis (Peritonitis ? ?)		
Total deaths	1		1		4		0

In the table each date corresponds to 1 dead animal. "Unknown" means in this connection that no postmortem examination was undertaken.

Table 6. Diseases affecting ER incidental to the experiment.

Animal No.	Disease	Group	Aberration of ER in period IV
69	Infected tail		— 0.80 mill.
76	Abscess	F_2P	— 0.21 »
77	Infected tail		— 0.27 »
82	»		— 0.28 »
84	»	F_2A	+ 0.11 »
96	»		— 1.70 »
99	»		— 0.33 »
156	»	F_2R	+ 0.28 »
157	»		— 1.57 »

ER = Erythrocyte count. Aberration = the mean of the two in each animal observed aberrations from the normal value M_i during period IV.

ficiently extensive. Moreover, they may render valueless an ordinarily efficacious blood treatment. Infection of the tail is the only type of suppurative inflammation observed in this experiment. In taking the blood specimens a small piece of the tail was cut off so that the blood could flow freely and without the need of stasis, whereupon the wound was coagulated with a searing instrument. This method is essentially very good but it is not absolutly infection-proof. In 8 of the 140 animals living at the end of the experiment a cut in the tail demonstrated the presence of much pus and for a long time before being killed 1 animal had a large infected sore on the left buttock. In the table below (6) the infected animals have been entered and the mean aberration of the erythrocyte numbers from the normal value are given for period IV. Evidently such infections were only encountered in groups F_2P, F_2A and F_2R and the proportions between the erythrocyte numbers make it clear that the inflammations were not without deleterious effect. A reduction has occurred which distorts means for the groups in question (see p. 51). 7 of the 9 animals responded with a reduced erythrocyte count. The probability that this should be a random effect is only 0.09. With the knowledge in view which we already have as to the effect of some infections on the blood picture, the tail infection in the present experiment very likely can be attributed to the reduction in red blood corpuscles.

In addition to the matter discussed above, nothing except the treatment has been observed which could conceivably have a systematic influence on the erythrocyte numbers. Nothing was noted indicating toxic action of the injection preparations.

III. Results

a. The Erythrocyte Count in the Various Groups before Treatment

What happened to the erythrocyte numbers during the time of observation? In order to adequately answer this question an expression must first be obtained for the normal (i. e. uninfluenced by injections) erythrocyte number of each animal. This figure has been arrived at by calculating the mean (M_i) for each animal of the 5 observations (x) during the normal period. In consequence hereof M_i will henceforth be called the *normal value*. In the following all individual aberrations of the erythrocyte number will accordingly be calculated from the *normal value*.

The individual x-values vary from one observation to the next. This is partly due to true variation and partly to error of method. The M_i-values are therefore also somewhat uncertain. The mean error for M_i may of course be calculated starting from the 5 x-values, but that method is cumbersome if it is to be applied throughout and the thusly calculated individual mean errors will moreover be very uncertainly determined. Assuming, on the other hand, that the individual variation actually is approximately the same for all the animals, we can however avail ourselves of an averaged expression for the mean error, which is obtained by a simple application

Table 7. **All x and M_i values during the normal period.**

ER[1]	x	M_i	ER[1]	x	M_i	ER[1]	x	M_i
	Frequency			Frequency			Frequency	
4.9	1	—	7.4	19	3	9.3	6	2
5.5	1	—	7.5	20	7	9.4	5	—
5.7	1	—	7.6	35	6	9.5	4	1
5.8	—	—	7.7	35	10	9.6	7	—
5.9	—	—	7.8	32	9	9.7	2	—
6.0	1	—	7.9	42	6	9.8	4	—
6.1	3	—	8.0	43	13	9.9	4	—
6.2	2	—	8.1	41	18	10.0	5	—
6.3	2	—	8.2	44	11	10.1	2	—
6.4	3	—	8.3	43	15	10.2	—	—
6.5	5	—	8.4	65	10	10.3	—	—
6.6	1	—	8.5	45	8	10.4	—	—
6.7	4	—	8.6	44	5	10.5	1	—
6.8	8	—	8.7	35	5	10.6	—	—
6.9	9	1	8.8	29	9	Total	770	154
7.0	9	1	8.9	28	3	Mean	8.210	8.203
7.1	11	1	9.0	22	2	Variation	0.5162	0.2418
7.2	15	2	9.1	11	3			
7.3	13	2	9.2	8	1			

[1] ER = Erythrocyte number. 4.9 comprises the values 4.90—4.99 etc.

of the so called analysis of variance (see appendix 1 p. 63). Since the total number of animals is 154 we get all in all 154 M_i-values and $5 \times 154 = 770$ x-values. If the variance in these two series is denoted by σ^2_M and σ^2, respectively, the required averaged mean error is expressed by the formula:

$$\varepsilon\,(M_i) = \tfrac{1}{2}\sqrt{\sigma^2 - \sigma^2_M}$$

σ and σ_M are calculated from a table 7 p. 36 showing frequency distributions of x and M_i values for all the 154 animals on which 5 observations were made before the start of the treatment.

$$\sigma^2 = 0.5162 \qquad \sigma = 0.72$$
$$\sigma^2_M = 0.2418 \qquad \sigma_M = 0.49$$
$$\sigma^2_v = 0.2744 \qquad \sigma_v = 0.52$$
$$\bar{\sigma}^2_v = 0.3430 \qquad \bar{\sigma}_v = 0.59$$
$$\varepsilon(M_i) = 0.26$$

Thus, the variation in the individual daily observations is however considerable (dispersion is calculated to in average 0.59). Hereby also the M_i-values will suffer

Table 8. Daily and period means of erythrocyte numbers observed before treatment in the various groups.

Daily mean per obs. day No.	Group							
	F_0	F_2	F_2M	F_2P	F_2A	F_2AK	F_2K	F_2R
1	8.70	8.39	8.02	8.24	8.00	8.38	7.98	8.08
2	8.62	8.23	8.05	8.50	8.02	7.95	7.87	8.05
3	8.52	8.32	7.79	8.45	8.27	7.97	8.14	8.30
4	8.35	8.33	8.23	8.32	8.08	8.21	8.05	8.22
5	8.62	8.40	7.89	8.29	8.13	8.05	7.86	8.14
Period mean	8.56	8.33	8.00	8.36	8.10	8.11	7.98	8.16

from great uncertainty; the mean error amounts to, as we see, 0.26. In unfavourable cases, therefore, the error in the individual 5-day means may be as much as 0.8 (3 times the mean error). This implies that during the treatment any weak tendencies to a change in the erythrocyte number can be obscured by large irregular random variations. To some extent, however, these can be eliminated and this will be explained below.

The next point of interest is to follow the erythrocyte numbers in the animals during the *normal period*, i. e. before the treatment. The values (x) from the individual animals vary greatly. Therefore a so called *daily mean* was calculated for each group, the daily mean being based on all 20 observations (x) made on the same day of observation in the respective groups.

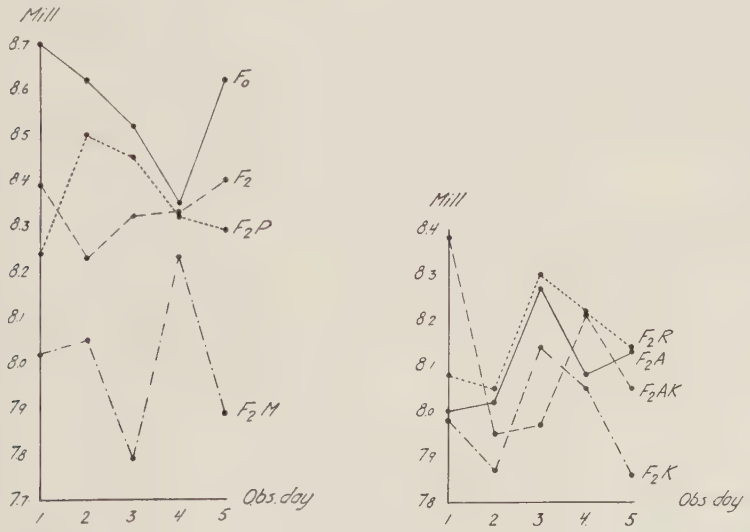


Diagram 6. The mean of all erythrocyte numbers for each observation day (daily mean) in each group during the normal period.

From the 5 observations carried out during the first 2 weeks about 100 x-values were obtained in each group. From these were then calculated so called *period means*.

On the basis of these means table 8 and diagram 6 were made up. From these it appears that during the normal period there was no definite tendency to change in the erythrocyte numbers. In part the different groups lie on different levels. Obviously there is no significant difference between the various period means. The group F_0, however, has the period mean 8.56 mill. which is rather more than could be expected from random variations alone. In the following will be demonstrated that this circumstance is of no significant importance in the manner of reacting to the treatment.

b. The Erythrocyte Count in the Various Groups during Treatment

As described above a so called normal value, here labelled M_i, was calculated representing the mean of the erythrocyte numbers of the first 5 observations. During this time (the normal period) no injections were given.

The daily values observed thereafter were compared with the M_i of the animal in question and the difference or aberration from M_i were recorded in so called working tables (see p. 64). From these tables daily aberrations the so called daily means — given in table 9 — were calculated for each group of animals.

If these figures are plotted in a diagram (7) we see that the individual curves are uneven and jerky because of great variations from one day of observation to the next. However, already at this early stage of the statistical treatment it is possible

Table 9. **Daily means of the aberrations of the erythrocyte numbers from the normal value during the periods of treatment. The minus sign indicates an observed reduction. Values in mill.**

Observation day after start of treatment	Injection groups							
	F_0	F_2	F_2M	F_2P	F_2A	F_2AK	F_2K	F_2R
1	0.38	0.16	0.09	0.17	0.24	0.03	0.27	0.03
2	0.47	0.02	0.19	—0.21	—0.14	—0.23	0 22	0.22
3	0.49	0.24	—0.11	0.05	—0.06	0.53	—0.45	—0.37
4	0.21	0.25	0.17	0.25	0.34	0.17	—0.14	0.16
5	0.18	0.16	0.00	—0.03	0.51	0.38	0.28	0.02
6	0.46	0.27	0.10	0.43	0.36	0.17	0.00	—0.24
7	0.26	0.29	—0.03	0.15	0.51	0.33	0.03	0.06
8	0.63	0.85	0.34	0.69	0.75	0.86	0.36	0.02
9	0.56	0.48	0.19	0.54	0.18	0.58	0.22	—0.06
10	0.59	0.54	—0.04	0.37	0.26	0.49	0.33	—0.15
11	0.78	0.84	0.32	0.35	0.21	0.73	0.23	—0.20

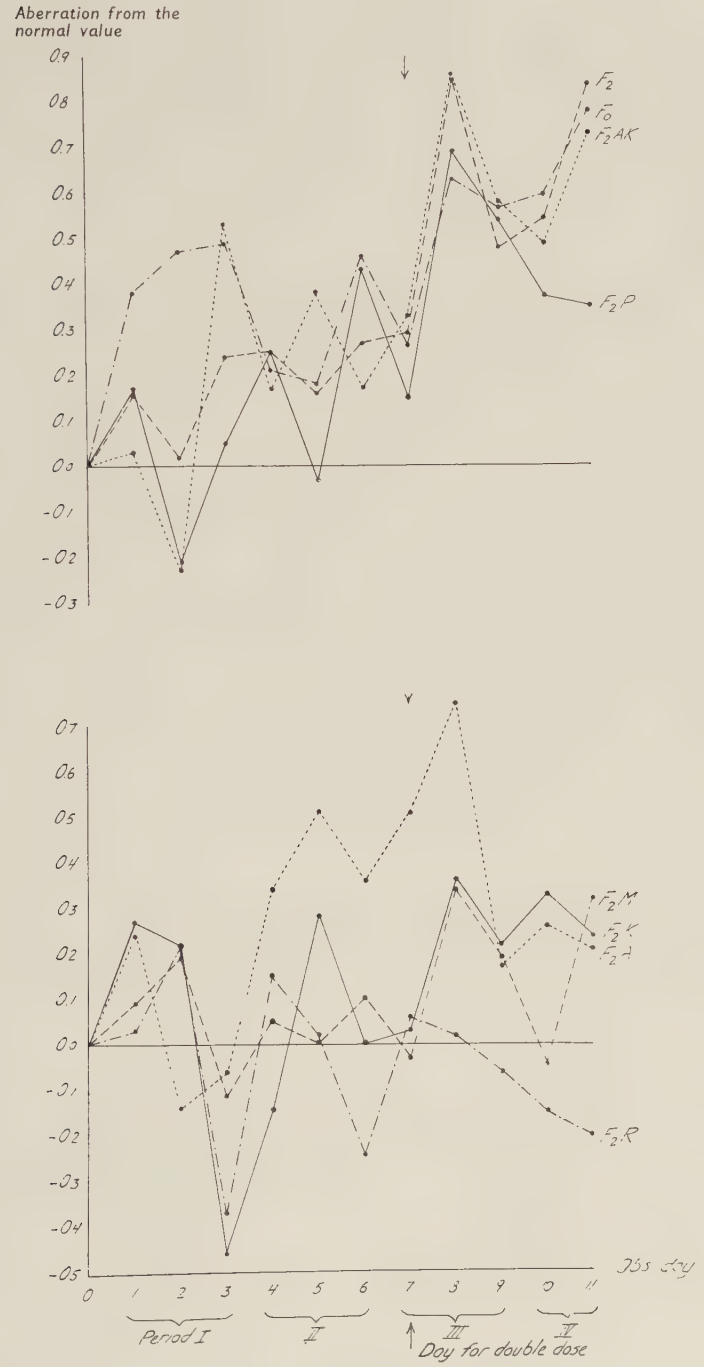


Diagram 7. Daily means of the aberrations of the erythrocyte numbers from the normal values during the period of treatment.

to discern certain tendencies. At the 8th. observation only the fractions F_2M, F_2K and F_2R still exhibit comparatively low daily means. In order to reduce the influence of random elements the means of the aberrations were now calculated for several days of observation lumped together. For this reason the time of observation was divided in to the periods I—IV (see p. 33). The periods were so chosen that during periods I—II the animals received a daily injected dose of 0.25 ml of the respective preparations and during periods III—IV 0.5 ml (see diagram 7).

The values thus obtained were tabulated (table 10).

A significant increase in the number of erythrocytes could, as seen in table 10, only be observed in the fractions which have been taken together under the heading, Group 1. From the manner of producing the preparations we know of these fractions that they certainly contain more batyl alcohol than F_2M and F_2K. If at this early stage we should run the risk of hypothesizing, that the observed increase in the number of erythrocytes is due to the batyl alcohol, it consequently follows that F_2R should be included in Group 2. Certainly this seems most likely considering both the relatively small yield of the extraction from red bone marrow and the complete absence of noticeable increase in the erythrocyte number in this group, which, judging by results, cannot contain any erythropoietic factor.

On the basis of table 10 diagram 8 was then made up demonstrating the change in the period means during the treatment. The statistically significant means are in the diagrams distinguished by a small circle. The remarkable thing is the no longer observable increase in period IV, fractions F_2A and F_2P. These observations will not be particularized at present, the author shall return to them in the Discussion (see p. 51).

We have now seen how the different injection groups behave separately during the

Table 10. **The numeric increase in red blood corpuscles (mean + standard error of the mean) in the various fractions during periods I—IV. Values in mill.**

Fraction	Period			
	I	II	III	IV
	Group 1. Fractions rich in batyl alcohol			
F_0	0.45±0.09	0.28±0.14	0.49±0.08	0.65±0.13
F_2	0.14±0.09	0.23±0.11	0.54±0.09	0.69±0.11
F_2A	0.03±0.13	0.40±0.13	0.48±0.13	0.23±0.15
F_2AK	0.10±0.09	0.24±0.12	0.59±0.13	0.61±0.08
F_2P	0.00±0.15	0.18±0.16	0.46±0.15	0.36±0.24
	Group 2. Fractions poor in batyl alcohol			
F_2M	0.06±0.11	0.09±0.13	0.17±0.17	0.14±0.15
F_2K	0.01±0.11	0.04±0.15	0.20±0.15	0.28±0.16
F_2R	—0.04±0.11	—0.02±0.18	0.01±0.17	—0.17±0.25

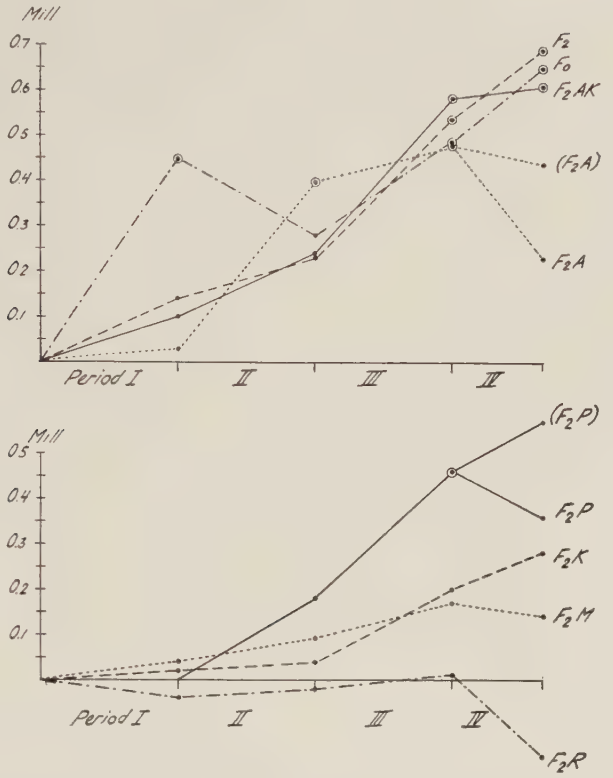


Diagram 8. Period means of the aberrations of the erythrocyte numbers from the normal values in the various groups during the period of treatment. O = statistically significant. (F_2A) and (F_2P) = the course of the curve if the diseased animals are excluded in period IV.

time of treatment. The batyl alcohol containing preparations were the only ones to give statistically significant increases. Therefore it is naturally interesting in connection with further testing of the supposed effect of batyl alcohol to amalgamate, on the one hand, the fractions given in table 10 under the heading Group 1 and those labelled Group 2, on the other hand. In effect this amounts to a comparison between the batyl alcohol containing preparations and those containing no batyl alcohol. The results are given in table 11.

Table 11. The mean increase in mill./c. mm. of the number of erythrocytes after amalgamation of the animals into a Group 1 treated with preparations rich in batyl alcohol and a Group 2 treated with preparations poor in batyl alcohol.

Period	Group 1		Group 2		Difference 1—2
	No. of animals	$M \pm \varepsilon(M)$	No. of animals	$M \pm \varepsilon(M)$	$D \pm \varepsilon(D)$
I	99	0.14±0.05	57	0.01±0.06	0.13±0.08
II	94	0.26±0.06	56	0.03±0.09	0.23±0.11
III	91	0.51±0.05	53	0.12±0.09	0.39±0.11
IV	89	0.52±0.06	52	0.06±0.12	0.46±0.13

Table *12.* Distribution of the individual aberrations of the erythrocyte numbers from the normal value in the various groups during periods I—IV.

	Aberration in mill.	F_0	F_2	F_2M	F_2P	F_2A	F_2AK	F_2K	F_2R
	Period I								
Decrease	1.51—2.00	—	—	—	—	—	—	—	—
	1.01—1.50	—	—	—	1	1	—	—	—
	0.51—1.00	—	1	2	4	2	1	3	3
	0.01—0.50	3	7	4	4	5	8	7	8
Increase	0.00—0.49	6	8	8	8	9	6	6	5
	0.50—0.99	8	4	3	1	2	5	4	4
	1.00—1.49	2	—	—	2	1	—	—	—
	Surviving animals	*19*	*20*	*17*	*20*	*20*	*20*	*20*	*20*
	Period II								
Decrease	2.01—2.50	—	—	1	—	—	—	—	1
	1.51—2.00	—	—	—	—	—	—	1	1
	1.01—1.50	1	—	1	2	1	1	1	—
	0.51—1.00	—	1	1	1	—	—	1	2
	0.01—0.50	2	5	4	3	3	4	5	3
Increase	0.00—0.49	6	8	5	7	6	8	7	8
	0.50—0.99	8	5	5	5	6	6	5	5
	1.00—1.49	—	1	—	1	3	—	—	—
	Surviving animals	*17*	*20*	*17*	*19*	*19*	*19*	*20*	*20*
	Period III								
Decrease	1.51—2.00	—	—	—	—	—	—	1	1
	1.01—1.50	—	—	1	—	—	—	—	2
	0.51—1.00	—	—	1	—	1	—	1	1
	0.01—0.50	1	2	4	4	2	3	1	3
Increase	0.00—0.49	8	8	6	5	8	5	10	8
	0.50—0.99	7	7	2	4	5	7	2	4
	1.00—1.49	1	3	2	2	3	3	2	1
	1.50—1.99	—	—	—	1	—	—	—	—
	2.00—2.49	—	—	—	—	—	1	—	—
	Surviving animals	*17*	*20*	*16*	*16*	*19*	*19*	*17*	*20*
	Period IV								
Decrease	3.01—3.50	—	—	—	—	—	—	—	1
	1.51—2.00	—	—	—	—	1	—	—	2
	1.01—1.50	—	—	—	—	—	—	—	—
	0.51—1.00	—	—	3	3	—	—	2	1
	0.01—0.50	2	—	4	3	5	—	5	5
Increase	0.00—0.49	5	9	4	2	6	7	3	6
	0.50—0.99	7	6	4	3	6	9	4	2
	1.00—1.49	2	4	1	1	1	3	2	3
	1.50—1.99	1	—	—	2	—	—	—	—
	2.00—2.49	—	1	—	—	—	—	—	—
	Surviving animals	*17*	*20*	*16*	*14*	*19*	*19*	*16*	*20*

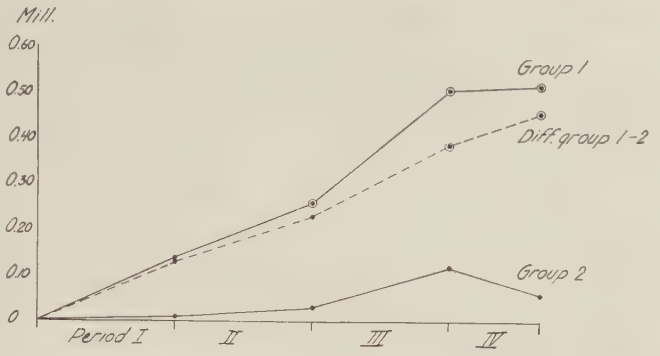


Diagram 9. The mean increase of the erythrocyte numbers (period means) in Group 1 (treated with preparations rich in batyl alcohol) and Group 2 (preparations poor in batyl alcohol) and the difference Group 1—2. O = statistically significant.

The difference between Group 1 and Group 2 is statistically significant in periods III and IV proving that the animals treated with preparations rich in batyl alcohol do not respond like the other animals, at any rate if due regard is taken to the mean increase in the number of erythrocytes. The table is reproduced graphically in diagram 9.

It is also interesting to find out how the various animal groups differ from each other systematically with respect to the number of animals exhibiting an increase during the time of treatment. In so doing the distribution of the aberrations from the normal values must first be ascertained. Starting from the working tables (see p. 64) we constructed the distribution table 12.

From this table it is easy to ascertain how many animals in each group that during periods I—IV exhibit an increased erythrocyte number in proportion to the total number of observed animals in each group. The results of this operation were tabulated (table 13).

Now, supposing we wish to ascertain in the simplest way whether there is an increasing or decreasing tendency in the erythrocyte count, we may proceed as follows: If there were no such tendency the increases and decreases could be expected to be just about as common, with due regard taken to random factors. The experiments would be (if regard were taken only to the sign of the change, not its magnitude) comparable to a game of "heads and tails". We may for example chose F_0, period I.

Table 13. **The number of animals exhibiting an increased erythrocyte number in proportion to the total number of observed animals in each group. Bold type = almost statistical significance. Italics = statistical significance.**

Period	F_0	F_2	F_2M	F_2P	F_2A	F_2AK	F_2K	F_2R
I	*16 : 19*	12 : 20	11 : 17	11 : 20	12 : 20	11 : 20	10 : 20	9 : 20
II	**14 : 17**	14 : 20	10 : 16	13 : 19	**15 : 19**	14 : 19	12 : 20	13 : 20
III	*16 : 17*	*18 : 20*	10 : 16	12 : 16	*16 : 19*	*16 : 19*	**14 : 17**	13 : 20
IV	*15 : 17*	*20 : 20*	9 : 16	8 : 14	13 : 19	*19 : 19*	9 : 16	11 : 20

Table 14. The percentage of animals that in relation to the normal value exhibited an increased erythrocyte number.

Period	Group 1			Group 2			Difference 1—2 in percent
	No. of animals	Whereof incrd.		No. of animals	Whereof incrd.		
		No.	%		No.	%	
I	99	62	62.6±4.9	57	30	52.6±6.6	10.8±8.2
II	94	70	74.5±4.5	56	35	62.5±6.5	12.0±7.9
III	91	78	85.7±3.7	53	37	69.8±6.3	15.9±7.3
IV	89	75	84.3±3.9	52	29	55.8±6.9	28.5±7.9

Out of a total of 19 animals 16 exhibited an increased erythrocyte number, whereas the "expected" number is 9.5 (19: 2). Calculations as to probability show that the figure 16 cannot very well have come about by chance.[1] We can therefore safely maintain that there is a systematic increase. This conclusion is so much more justified as the same tendency predominates with regard to periods II—IV. By means of this elementary method a systematic increase can also be demonstrated in the following cases:

F_2 period III—IV
F_2A » III
F_2AK » III—IV

There consequently is a systematic difference, which as a rule appears during the latter stages of the treatment, between the groups just mentioned and the other groups in the experiment. The only reason for this phenomenon that with certainty can be proved is the dissimilarity in treatment, i. e. in the injection preparations. The groups which were treated with preparations rich in batyl alcohol contained the largest number of animals with positive aberration from the normal value of the erythrocyte number. (The F_2P and F_2A groups are exceptions. It can, however, be demonstrated that this is due to diseased animals that do not react like the other animals. The connection between the disease and the comparatively low blood value can be proved as appears from the discussion p. 51.)

If we now on the basis of the data given above wish to test the truth of the hypothesis, that the quantity of batyl alcohol administered is of importance for the observed differences, the simplest way is to proceed as is described on p. 41 and amalgamate the batyl alcohol containing series into a Group 1 and the other series to a

[1] The material is too small to permit common standard mean error analysis. Probability calculations were instead used according to the formula

$$\binom{n}{r} p^r q^{n-r} \text{ or in this case } \binom{n}{r} \frac{1}{2^n}$$

For $n = 19$ and $r = 0, 1, 2, 3, 16, 17, 18, 19$ we obtain the total probability = 4.4 ‰, i. e. exceedingly small if the result only depends on chance.

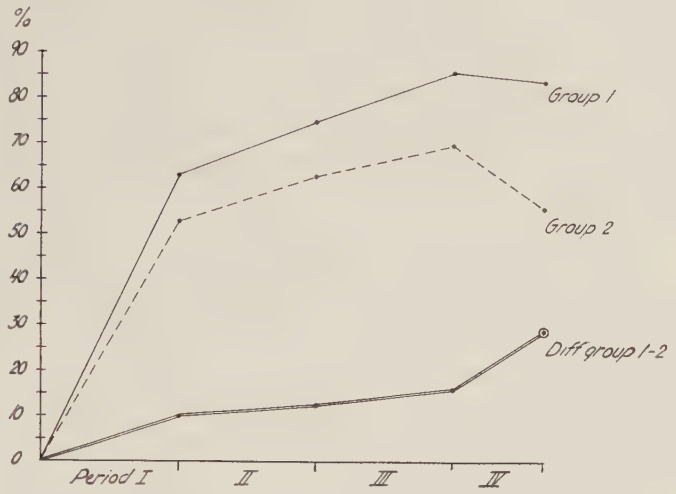


Diagram 10. The percentage of animals in Groups 1 and 2 that during periods I—IV exhibited an increased erythrocyte number. Difference Group 1—2. O = statistically significance.

Group 2. The two groups thus obtained are then compared in such a manner that from the preceding table 13 is calculated the number of animals exhibiting an increased erythrocyte number in relation to the normal value. This number is then expressed as a percentage for each period and group of the total number of animals observed. The results are collected in table 14.

In period IV it appeared that 9 animals were infected (see p. 34). Of these 7 belonged to Group 1 (F_2P and F_2A) and 2 to Group 2 (F_2R). If these animals are neglected in the calculations for that period the figures for Group 1 will be 90.2 $\pm$ 3.3 % (74 out of 82) with increased erythrocyte number and for Group 2 56.0 $\pm$ 7.0 % (28 out of 50). The difference between these percentages is 34.2 $\pm$ 7.8 and is consequently statistically significant.

The percentages obtained are reproduced graphically in diagram 10.

From table 14 and diagram 10 it is evident that the difference between Group 1 and Group 2 with respect to the number of animals exhibiting an increased erythrocyte number rises continually and in period IV it is statistically significant. By means of these two methods of treating the material an agreement has thus been demonstrated between both the mean increase of the erythrocyte number and the number of animals with increase. Both methods gave as a result that there is a systematic difference between the animals treated with preparations rich in batyl alcohol and those given substances poor in batyl alcohol.

IV. Discussion

In the foregoing it has been shown that in this animal experiment only those individuals exhibited a significant increase in the number of red blood corpuscles that had received injection preparations which with certainty contained batyl alcohol, viz. F_0, F_2, F_2P, F_2A and F_2AK.

Substances containing little or no batyl alcohol gave no significant increase in the erythrocyte number.

The observed increases in the erythrocyte number have by statistical methods been shown to be systematical and chance or random elements can therefore be excluded right away. Consequently the only remaining reasons for an increase, apart from the treatment are the following:

1. There is an initial systematic difference in the various animal groups.
2. Systematically acting factors unconnected with the treatment have at some time during the observation period happened to influence one or more groups.
3. Common to all the animal groups there has been a tendency towards increase in the erythrocyte number, although in some groups it has been inhibited, i. e. the groups F_2R, F_2M and F_2K. Then also the sesame oil might have contained inhibitory or hemotoxic factors. A general increasing tendency could conceivably be due to the following circumstances:

a. A stimulation of the bone marrow through repeated small blood losses.
b. A seasonal variation.
c. The erythrocyte number increases with mounting age of the animals.
d. The sesame oil causes the increase.

Ad 1. During the first two weeks of the experiment nothing was done to the animals except taking a blood specimen every 3rd.—4th. day. In this period no specific changes could be observed in the erythrocyte count (see table 8 p. 37 and diagram 6 p. 37). Moreover the animals were of the same strain and age and there was a like number of males and females. In the first selection all animals with external signs of disease were naturally discarded. There consequently was no initial difference of importance in this connection in the animal groups.

Ad 2. Except for the composition of the injection preparations all the animals partaking in the experiment were treated exactly alike. No change in method except for the doubling of the administered dose — which took place simultaneously throughout — was undertaken after the experiment had started. As is described on p. 34 a small number of animals became ill during the time of observation but the effects of the diseases were such that the hypothesis as to the beneficial action of the treatment sooner is supported than otherwise. Therefore the subject will not be discussed here but on p. 51. During the time of observation the author was unable to observe any systematically acting circumstance that could in any way explain the differences in behaviour between the animal groups.

Ad 3 a. By increased function of the red bone marrow the organism tends to compensate for the loss in erythrocytes resultant of blood losses so that when the blood loss is continuous there will at best be a state of equilibrium — a balance between the blood loss and the blood regeneration. In such cases the organism as a rule conditions itself to a subnormal erythrocyte number. (Whitby and Britton.)

The author has for a period of two months observed this phenomenon in a group

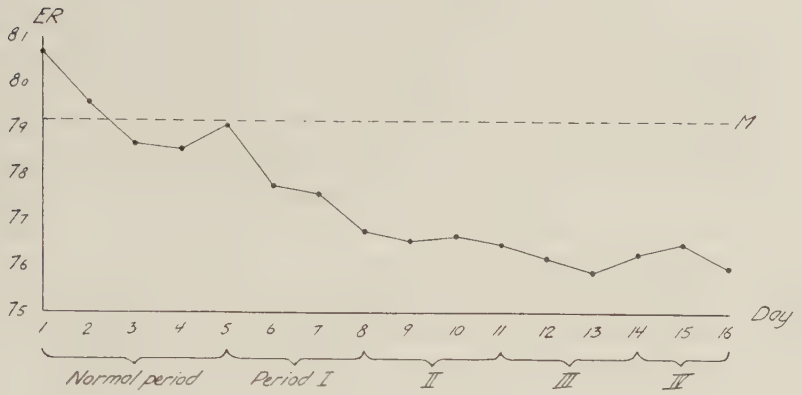


Diagram 11. Daily mean of the erythrocyte numbers in 60 albino rats in which bloodsampling only was carried out every 3rd—4th day for 2 months. ER = erythrocyte number in mill/c. mm. M = mean of the first five observations. Day = observation recurring every 3rd—4th day.

of 60 albino rats in which bloodsampling only took place every 3rd—4th day. This experiment which was undertaken one year later during the same months was in all essentials identical to the experiment under discussion save that no injections were given and incondsiderably more blood was taken. If the daily means for the 60 animals are tabulated (diagram 11) no change can be seen during the latter half of the observation time. No increase in the erythrocyte number, which could characterize a more active bone marrow function, can be observed. It does, on the other hand, seem as though a balance had set in between loss and regeneration of blood. Although this experiment cannot be numerically compared to the previous one it does show the tendency in the reaction of the animals to a small continuous loss of blood: first a reduction in the erythrocyte number, then stabilization for a varying length of time at a certain subnormal level. With this in view the supposition is absurd that a small continuous loss of blood should cause an increase in the erythrocyte count. It would be an inadequate response from the organism to an adequate bone marrow stimulation.

Ad 3 b. Is it possible there was a seasonal variation so that at the start of the experiment all the animals had a spontaneous tendency to a slow increase in the erythrocyte numbers?

That such a tendency is *not* present as from the beginning of the time of observation is fully evident from experimental data already given. Moreover, in this case the seasonal variation could not have set in until the start of the treatment as it could not be observed earlier and in addition it must have started at different times in the various groups of animals. In fact, they were all treated and observed for the same length of time with a phase difference of at most four days among the animals.

STARKENSTEIN discusses seasonal variations in the blood picture of the rabbit and dog. His investigations did not cover the months July and August and his

results are mutually incompatible. For rabbits he reports a mean erythrocyte number for June and September of 4.96 and 5.86, respectively, and for dogs 6.96 and 6.93 mill. respectively.

Whether or not seasonal variations do occur in albino rats is at the moment of writing unknown. Neither DONALDSON nor GRIFFITH and FARRIS have anything to say on the subject. It is not impossible, however. BENEDICT and MACLEOD have demonstrated that during the summer months the production of heat of the albino rat is approximately 10 percent less than during the winter. Theoretically, a slightly lower summer than winter value is, of course, conceivable.

At different times of the year the author has initiated experiments with rats from the same strain as those in the present investigation. The animals had all been born and kept in the same cellar, the food, bloodsampling technique, etc. have been identical and therefore a seasonal variation, which of necessity must be a constant phenomenon, should have been apparent. A comparison of normal values from these different experiments (only those comprehensive enough to permit of statistical treatment) yields the following results:

Month	No. of animals	Erythrocyte number
February	32	8.36 ± 0.19 mill.
July	154	8.20 ± 0.04 »
Sept 25th	27	8.16 ± 0.27 »

Here the winter figures are perhaps highest. The difference is, however, not statistically significant. Through July—September there is no change whatsoever. The experiment under discussion embraces the months July and August.

In consequence hereof the author finds no reason to calculate with or suspect the presence of any seasonal variation during this time of observation.

Ad 3 c. During the growth to maturity of the albino rat the number of erythrocytes increases rather rapidly up to an age of 90 days. Thereafter the increase is extremely slow indeed. DONALDSON has put the figures at 8.5 and 8.9 mill. at an age of 90 and 1095 days, respectively. The increase that might take place during two months in the rats observed by the author in this experiment, all of which were more than 6 months old, is therefore unimportant.

Ad 3 d. In order to answer the question regarding the injected sesame oil and whether it can be held responsible for the observed increases in the erythrocyte numbers of some animals, a special experiment was carried out in immediate connection with the investigation under discussion.

7 albino rats of the same strain as the other animals were subjected to exactly the same treatment as those given bone marrow extracts, with the single exception that they were given pure sesame oil only in the same manner and at the same dosage as the other animals.

The normal values of the 7 animals varied between 7.21 and 8.48 mill., and the mean was 7.82 mill.

In order to estimate the change in the erythrocyte number during the period of treatment the mean of the four last observations was calculated for each animal. Thereafter the difference between this mean and the normal value was registered for each individual animal. The mean deviation from the normal value was — 0.26 ± 0.20 mill. Therefore the erythrocyte number decreased from 7.82 to 7.56 mill. during the treatment. As we see the decrease is not statistically significant. If the animals are taken separately we find an increased erythrocyte number in 3 animals and a decreased in 4 animals. The greatest increase was only 0.28 mill. Although no definite statement as to the action of the sesame oil can be formulated on the basis of this experiment, it does seem probable that sesame oil alone cannot cause any significant increase in the erythrocyte number. It is, however, possible to arrive at a definite opinion as to whether the increase in the erythrocyte number observed in, say, the F_2AK group is caused by the sesame oil or not.

The 19 animals that were given injections of F_2AK dissolved in sesame oil were in other respects treated just like the 7 animals mentioned above which latter had received sesame oil only. For every single one of the 19 animals was calculated the difference between the normal value of red blood corpuscles and the mean of the last four observations during the period of treatment. In all 19 animals there had occurred an increase in the erythrocyte number. In 4 cases the increase amounted to 1 mill. and in 6 instances less than 0.5 mill. per c. mm. The mean increase was 0.65 ± 0.09 mill. and is statistically significant. A tabulation of the results obtained from the two series of experiments follows below:

A. *Sesame oil + F_2AK (19 animals)*

Mean of normal blood value (before treatment)	8.13
» » blood value at end of treatment	8.78
» » the difference of 19 animals	+ 0.65 ± 0.09

B. *Sesame oil (7 animals)*

Mean of normal blood value before treatment	7.82
» » blood value at end of treatment	7.56
» » the difference of 7 animals	— 0.26 ± 0.20

Owing to the fact that the number of animals in the two compared series is small it is necessary to employ a special statistical method (introduced by W. S. Gosset and R. A. Fisher):

We assume that the fraction F_2AK has no influence at all on the erythrocyte number and that the individual animals in the two samples react alike to the sesame oil action, if any. The two samples of 7 and 19 animals are therefore considered as taken at random from the same collective. In other words the two samples together are considered a homogeneous material of 26 animals. If this material is divided anyhow into two parts of 7 and 19 animals and the number of red blood corpuscles counted for each part the two means will differ more or less.

In the present instance our problem is: How great is the probability that the dif-

ference between the two means obtained in this manner will at least be as large as the difference between — 0.26 and + 0.65?

If the necessary numerical calculations are carried out we find that the probability for at least as large a difference ($\geqq 0.91$) is practically zero.[1]

This implies that our working hypothesis: that the two samples were taken at random from the same collective, is incorrect. The 19 animals in the first series have reacted during the treatment with an increased erythrocyte count in contradistinction to the 7 animals in the second series. The animals in the two series were observed just as long and absolutely alike except for F_2AK. This leads to the conclusion that the increases in the erythrocyte numbers must be attributed to the F_2AK fraction and not to the sesame oil.

Since the sesame oil in the cited example caused no increase in the number of erythrocytes it probably did not do so in the other series of animals as well. Consequently sesame oil does not increase the erythrocyte number.

Summing up we may now maintain that the only reasonable explanation for the behaviouristic differences observed in the animals must be differences in treatment. Some of the animals had exhibited more red blood corpuscles, others had not. The increase in the erythrocyte number must therefore be due to the fact that the respective preparations contain a sufficient quantity of one or more somehow erythropoietically active factors.

A significant increase in the number of erythrocytes was registered during treatment with the preparations F_0, F_2, F_2P, F_2A and F_2AK.

Common to these preparations is that they contain batyl alcohol and F_2AK is pure batyl alcohol. We know about the preparations F_2M and F_2K, which caused no significant increase of the erythrocyte number, that they in comparison to the other preparations can contain at most small quantities of batyl alcohol.

After the experiment with F_2AK we know that batyl alcohol increases the erythrocyte number. *Evidently the presence of sufficient quantities of batyl alcohol is essential for the action of the other preparations.*

Naturally, the possibility cannot be excluded that the nonsaponifiable fraction of yellow bone marrow contains other erythropoietically active substances, but the present investigation has yielded no results in proof of this.

Now it is easier to form an opinion of the F_2R preparation. It was produced from fat in red bone marrow. It is absolutely evident that this fat can contain no accumulations of erythropoietically active substances. Hence it follows that the preparation is poor in batyl alcohol and therefore it is fully justifiable to group it with the preparations poor in batyl alcohol in the statistical treatment. Additional support of this assumption is afforded by the relatively meagre yield of the substance obtained by the extraction.

[1] The test ratio (t) according to Fisher's significance test for small samples is in this case $t = 5.3$. At 24 degrees of freedom the probability is 0.001 that t will exceed the value 3.7. Practically speaking, therefore, the probability is zero that t will be greater than 5.

The experiment was not altogether ideal. In groups F_2P, F_2A and F_2R a number of animals were infected (see p. 34). That this infection had a probable connection with a reduction of the erythrocyte number has been shown on p. 35 and 45.

In table 10 p. 40, we see that the erythrocyte number for period IV decreases in groups F_2A, F_2P and F_2R. If the sick animals are disregarded in the calculations the following figures are obtained for the mean increase in period IV.

F_2P 11 animals $+ 0.57 \pm 0.27$ (14 animals $+ 0.36 \pm 0.24$)
F_2A 15 animals $+ 0.44 \pm 0.11$ (19 animals $+ 0.23 \pm 0.15$)
F_2R 18 animals $- 0.12 \pm 0.26$ (20 animals $- 0.17 \pm 0.25$)

Moreover, the F_2P group exhibits the highest mortality and during periods III and IV two animals died which during period III both had increased their erythrocyte counts.

Consequently there is a fully acceptable explanation of why the result of treatment with preparations F_2P and F_2A were not very successful during period IV.

V. Summary

By experiments on albino rats the author has demonstrated the following:

1. The nonsaponifiable fraction of yellow bone marrow fat from cattle has given a statistically significant increase in the erythrocyte number.

2. By repeated fractionation until a simple substance was arrived at, batyl alcohol, and testing of the fractions thus obtained, a similar action on the erythrocyte number has in some cases been recorded.

3. Statistically significant results have been obtained only in testing the fractions rich in batyl alcohol and batyl alcohol itself. Therefore the batyl alcohol can be assumed to be essential for the observed increase of the erythrocyte numbers.

Chapter V

EXPERIMENTS WITH HEALTHY HUMAN SUBJECTS

Since the batyl alcohol tested in previous experiments proved to be erythropoietically active in albino rats it would be interesting to learn if it has the same influence in man and an experiment was therefore carried out.

A. Material and Methods

It would have been desirable to test the optically active antipode of batyl alcohol — which was used in the animal experiments — but this encountered practical obstacles. I therefore had to remain content with a synthetic, optically inactive antipode of batyl alcohol dissolved in oleum olivae to a concentration of 10 mg/ml.

The test persons, 10 male medical students aged 19—21 years, were first observed for a period of one month, blood specimens being taken almost daily. Then each one was given a peroral dose of about 240 mg batyl alcohol in doses of 20 mg per observation day.

The treatment having been concluded the test persons were observed for rather more than two months. Then a new period of treatment was started with 45 mg per observation day so that each test person thus was given a total of 450 mg. An observation period of 14 days followed in immediate succession and the experiment was discontinued.

Hemoglobin contents, red and white blood cell counts, and reticulocyte and differential smears were studied for each test person.

The hemoglobin was determined by Sahlis' method.

The erythrocyte numbers were determined by drawing up blood into a Hellige pipette to the 0.5 graduation and then Hayem's solution to the 101 graduation. Then the specimen was counted in a counting chamber by filling both chambers from the pipette and always counting the same 8 CD squares. The values obtained were added. Hemoglobin determinations and erythrocyte counts were always done by the same person. Each test person had his personal, marked pipette.

The reticulocyte contents was determined by mixing on a slide a drop of blood and a drop of reticulocyte staining liquid (brilliant cresyl blue). The mixture was then allowed to stand for a minute or so before being smeared out. 1 000 cells were then counted in the microscope.

B. Results

Of immediate interest were reticulocytes, red blood cells and hemoglobin.

In order to eliminate to some extent the chance variations in the individuals a daily mean was calculated for the 10 persons. In a series of 16 observations before the two periods of treatment the mean and the standard deviation were calculated. Presupposing a normal distribution the highest daily mean at random is $M + 3\sigma$ where M signifies the mean of the 16 observations. The results of the investigation into the reticulocyte count are given in the below table.

	Before the period of treatment	
	I	II
Mean of 16 observations (days), M	7.0	7.2
Standard deviation, σ	1.12	0.90
Highest daily mean at random, $M + 3\sigma$	*10.4*	*9.9*
Highest observed daily mean	9.2	8.9

The first period of treatment (I) comprised about 14 days, i. e. from February 26th. to March 12th., inclusive. The second period (II) lasted from May 4th. to 14th., inclusive. Unfortunately it sometimes happened that all the test persons could not be treated on the same day. The following table therefore also gives the number of persons who on the various days during and immediately after the period of treatment are included in the calculations of the mean reticulocyte numbers. In this connection it is important to point out that the test persons who on some days could not be observed did not exhibit particularly low values. The persons whose values are included in the mean are in other words not a select group with high values.

During the 1st period of treatment.

Date	26.2	27	28	1.3	2	4	5	6	7	8	9	10	11	12
Ret. val.	7.5	7.3	12.9	11.7	11.6	11.3	13.1	14.7	13.9	13.3	14.1	13.3	16.4	16.7
No. of ind.	10	10	10	7	7	10	10	10	10	10	10	4	10	10

After the 1st period of treatment.

Date	13.3	14	15	16	17	18	19	20	21	22	23	24	25	26
Ret. val.	16.0	16.4	13.6	16.7	—	14.2	11.1	13.1	12.8	9.6	9.2	—	—	7.6
No. of ind.	9	9	10	6	—	9	9	9	9	9	10	—	—	9

From the above reproduced figures it plainly appears that an increase in the reticulocyte value sets in during the period of treatment. This increase cannot be a matter of chance. The highest permissible value at random is as we know 10.4 reticulocytes per thousand. During the first two days the value is "normal" and then it is significantly higher during the entire period of treatment. The highest observed value was for the last day of treatment (12.3). Then the value was 16.7 and the *dif-*

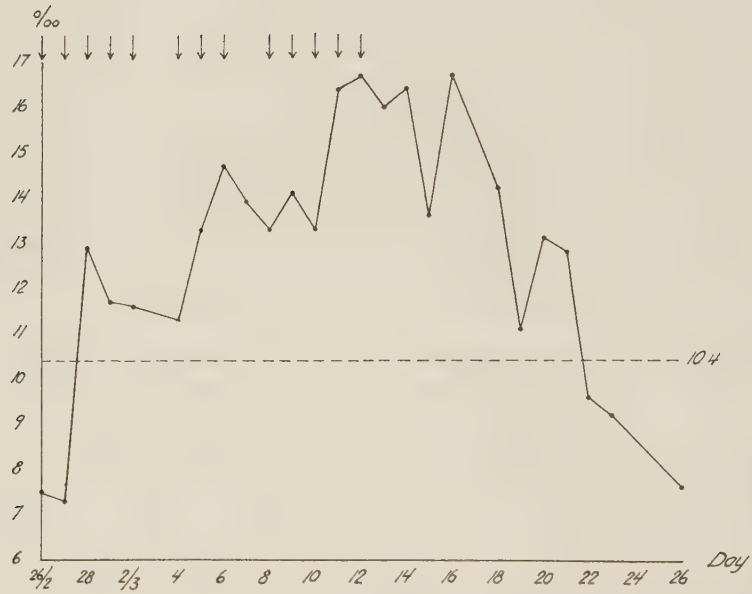


Diagram 12. Reticulocyte number (daily mean) during and after the first period of treatment of 10 young male adults. with daily (see the arrows) administration perorally of 20 mg. of synthetic, optically inactive batyl alcohol in olive oil. 10.4 = highest value at random before treatment.

ference between this value and the normal value 10.4 *is not less than almost 6 times the standard deviation.*

It is also interesting to note that the reticulocyte value remains on a higher level during 9 days *after* the end of the treatment. After that the reticulocyte value again becomes normal.

The second period of treatment which started on May 5th. gave the same result.

During the 2nd period of treatment.

Date	4.5	5	6	7	8	9	10	11	12	13	14
Ret. val.....	6.3	10.8	11.5	14.6	12.2	11.8	13.4	10.6	—	13.8	15.9
No. of ind.....	9	5	10	9	10	10	10	9	—	8	9

After the 2nd period of treatment.

Date	15.5	16	17	18	19	20	21	22	23	24	25
Ret. val.....	14.5	13.7	15.3	11.0	—	12.7	11.8	10.4	6.4	8.6	6.9
No. of ind.....	8	10	9	8	—	7	8	10	8	7	9

Since the highest permissible reticulocyte value at random is 9.9 the table above shows that an increased value sets in already on the second day of treatment and remains during the whole period and a further 8 days after the end of the treatment. Here also the last day of treatment exhibits the highest value. Then 15.9 per thousand

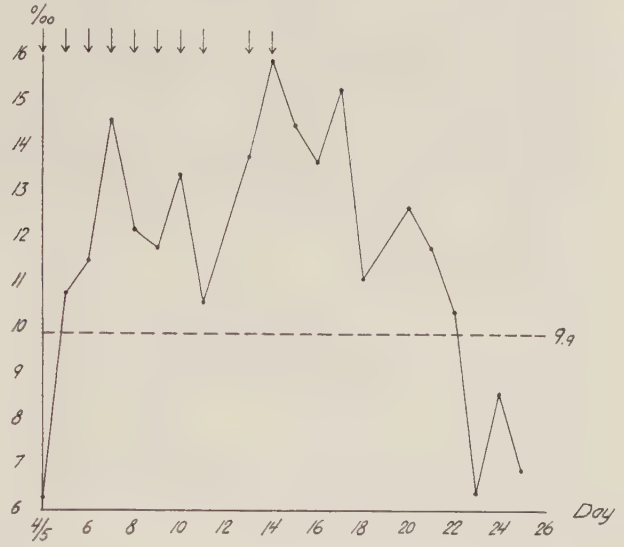


Diagram 13. Reticulocyte number (daily mean) during and after the second period of treatment of 10 young male adults with daily (see the arrows) administration perorally of 45 mg. of synthetic, optically inactive batyl alcohol in olive oil. 9.9= highest value at random before treatment.

reticulocytes were observed. *The increase* which is 6.0 *is well 6 times the standard deviation.*

The results are illustrated graphically in the diagrams 12 and 13.

Before the periods of treatment the mean erythrocyte number was 5.1 mill. for the 10 test persons. The standard deviation was 0.13 mill. and therefore the mean for one day during the treatment should exceed 5.5 mill. to be considered raised. During all the days of treatment this occurred only twice (5.52 and 5.55 mill.). The value being a mean of only 4 and 5 respectively of the 10 test persons, it can be given a limited weight only. Values of 5.35—5.40 occurred on 3 days.

The hemoglobin value was in mean 90.8 before the first and 87.6 (not corrected) before the second period of treatment. The standard deviation was 1.36 and 1.12 respectively. The highest permissible value at random was not exceeded at any time during the two periods of treatment or the time following immediately after.

C. Discussion

In the experiments described above was observed during and immediately after the periods of treatment a small but statistically significant increase in the number of reticulocytes and, furthermore, an insignificant increase in the erythrocyte number on two separate days of treatment.

The periods of observation before and after treatment were respectively 1 and 2 1/2 months. During these periods no significant spontaneous increase in the reticulocyte value was observed. It was consequently stable.

In immediate connection with the periods of treatment *all* the test persons ex-

hibited symptoms of reticulocytosis. It was observable after twenty-four or fourty-eight hours (24 hours during the 2nd. period of treatment). That the increase in reticulocytes is a direct consequence of the treatment is absolutely above every doubt.

The only essential difference possibly caused by the higher dosage in the second period was the more immediate response. A higher value was to be expected, but, since up to the present nothing whatsoever was known about the reticulocytosis promoting action of batyl alcohol or its dosage, the effect of the higher dosage could not at all be foretold.

During the 1st period of treatment, especially, the consistently increasing tendency of the reticulocyte number is plain and the highest daily values observed occur on the last day of treatment in both periods. This may, of course, be purely accidental but it might also indicate a certain cumulative effect of the batyl alcohol.

Numerically the reticulocytosis may seem to be slight but nevertheless it is what could be expected with an erythrocyte count around 5 mill. before the treatment. In hematology it is well known that in treatment the reticulocytosis almost always is in reverse proportion to the erythrocyte count at the start of treatment.

Nothing definite can be said about the erythrocyte number in this experiment. It cannot be said that the erythrocyte count did not increase (during the treatment higher values were actually observed than before or after). It may be asked if the increased number of reticulocytes could have appeared in the counting chamber as an increased number of erythrocytes, but the increase in reticulocytes observed here is too small to with certainty raise the erythrocyte count by more than 400 000 blood cells per c. mm. at least during the comparatively short duration of the treatment. To get certainty as to the behaviour of the erythrocyte number under batyl alcohol therapy further studies are therefore required.

It is intrinsically strange that the batyl alcohol at all affects the activity of the red bone marrow in man. That a change actually has taken place has been proved, and every reticulocytosis, even the "aspecific" one (reticulocytosis not followed by an increase in the erythrocytes), implies changed bone marrow activity.

Batyl alcohol was also used in the experiment with albino rats, but it was optically active and several instances from biochemistry tell us that a biological effect often is dependent on some optical activity of the substances tested. In this experiment on human subjects optically inactive batyl alcohol was used and consequently it is important for reasearch to proceed further along these lines using the optically active antipode. Other doses should also be tried.

D. Summary

On a series of 10 healthy men aged 19—21 the author has tested the effect of synthetic, optically inactive, perorally administered, batyl alcohol on the erythrocyte number, hemoglobin contents and the reticulocyte number in the blood both during and after two periods of therapy.

The blood forming mechanism was proved to be influenced in so far as a statistically significant reticulocytosis set in owing to the treatment. No definite conclusion could be drawn as to the erythrocyte counts. No changes could be observed in the hemoglobin contents of the blood.

A correct interpretation of the results and a further study into the action of batyl alcohol presupposes continued research.

Chapter VI

FINAL SURVEY OF RESULTS

A. Summary of Results

In Chapter IV B the author describes experiments with benzene depleted albino rats. It was found that animals which in addition to benzene were treated with the preparation F_0 (the unsaponifiable fraction of yellow bone marrow fat) exhibited

1. better general condition
2. lower death rate
3. higher erythrocyte count.

These differences were most evident only after some increase in the dose of F_0 administered.

In Chapter IV C the author describes experiments on otherwise untreated albino rats with the various bone marrow preparations accounted for in chapter III. The following results were obtained:

1. The preparation F_0 and
2. fractions of this preparation with exception of those poor in batyl alcohol
3. and by fractionation of F_0 obtained batyl alcohol, collectively augmented the erythrocyte counts.

In Chapter V the author describes an experimental series on human material with synthetic, optically inactive batyl alcohol yielding the results:

1. 20—45 mg of batyl alcohol per observation day given perorally in repeated doses augmented the number of reticulocytes in the blood
2. High erythrocyte counts were observed in connection with the treatment but could not be made statistically significant owing to the small size of the material.

B. Discussion of the Test Material

In his animal experiments the author used albino rats only. In hematologic studies the rat offers some advantages but also great disadvantages as a test material Among the advantages must above all be reckoned the simplicity of experimenting with a large number of individuals of the same stock. Another advantage is the comparatively great resistance to a number of deleterious infections that may de-

velope as a consequence of e. g. repeated intraperitoneal injections or superficial minor incisions to obtain blood specimens.

A disadvantage from the purely hematologic point of view is of course the considerable irregular random variations in the number of blood corpuscles. These variations, which can be either spontaneous or due to errors of method, entail the necessity of a very large material to get statistically significant results in demonstrating small systematic differences between the types of blood corpuscles most varying in number, e. g. reticulocytes and white blood corpuscles. In connection with an investigation of this type it would have been interesting to have available reliable information as to the behaviour of the reticulocytes under influence of the treatment given. Such figures do collected by the author, however, show that the variations from one animal to another are considerable. The very first blood specimen (taken before any treatment is given) from one animal may contain 3 and from another 60 promille reticulocytes. The variations in one and the same animal are, moreover, often of this magnitude which in its turn is irregular. The situation is equivalent as regards the white blood corpuscles and in this case also it is on the basis of the material studied impossible to draw any certain conclusions. However, in parentheses it may be mentioned that in the benzene experiment there was a significant difference in the total number of leukocytes in favour of the animals treated with the F_0-preparation.

The ability of batyl alcohol to increase the number of reticulocytes in the blood was, however, demonstrated by the author's experiments with human material.

In experimenting on albino rats it should be taken into account that the results may be influenced by diseases etc. In this connection it is of main interest to point out the occurence of blood parasites and then especially Bartonella Muris.

Bartonella Muris is a protozoa occurring all over the world and infesting rats, mice and other animals. In blood smears that have been stained in the usual way the disease can be demonstrated by the presence of coccoid or bacilloid bodies on or in the red blood cells. For each one of his animals the author has studied the red blood picture by e. g. differential counting and in one only of the benzene animals could he demonstrate manifest infection, despite the fact that the examiner's attention was directed to the possible presence of this blood parasite. This is probably due to the fact that in the albino rat Bartonella infection does not become manifest until after splenectomy (Griffith and Farris). Indeed, in the albino rat it can be induced experimentally by blocking the reticulo-endothelial system with e. g. Indian ink. One of the results of the manifest infection is an anemia developing within 3—16 days and becoming considerably pernicious, down to 1 mill. red blood corpuscles per c. mm.

Whether or not the Bartonella infection somehow can have influenced the erythrocyte numbers discussed in this work is naturally very difficult to tell with any degree of certainty, especially as the infection never became manifest. It is an open question if the treatment did have the effect of improving any blood disorder that

may have been caused by Bartonella muris. This eventuality is especially conceivable as regards the benzene depleted animals. In them there could have taken place an indirect action in so far as the reticulo-endothelial cells in the animals treated with F_0 had not been as badly damaged by the benzene. However, nothing indicates the existence of a direct action of the bone marrow extract on the Bartonella. The fact that in the experiment with human material a certain reaction to the treatment can be demonstrated makes rather far fetched the possibility of an action through the mediation of Bartonella Muris.

C. Summarizing Discussion of Results

Thus, it has been shown that F_0 (the nonsaponifiable fraction of yellow bone marrow fat) has a beneficial action on the erythrocyte number both in benzene depleted and untreated animals.

The chemical composition of F_0 has not been unravelled nor was it made a target in this work. All we know is that F_0 contains batyl alcohol and sterines with the possible addition of other nonsaponifiable fats. All free fatty acids, neutral fats and water soluble substances are removed.

All that is known with certainty as regards benzene depletion are the consequences. We do not know whether the benzene causes the demonstrable damages in the form of C_6H_6 or as, e. g., an oxidation product. Therefore it is at present impossible to explain the action of F_0 on benzene depleted animals. However, several reasons (see p. 31) indicate that there must be some sort of antitoxic effect. Whether this is effected by direct binding of the benzene ring to some component in the extract or by an altered permeability in the lipid membranes of the respective cells I cannot definitely say.

Continued experimentation with F_0 resulted in increased erythrocyte numbers in healthy animals also, and, for previously mentioned reasons, the author attributes this to its contents of batyl alcohol.

These latter experiments suggest that in one way or another the batyl alcohol dissolves in the lipid membrane of the erythroblast and directly or indirectly exerts an activating effect resulting in an increase in the number of erythrocytes and reticulocytes. Further studies are necessary to prove the correctness of this hypothesis.

The fact that the author has limited himself to a discussion of the erythrocyte numbers only does not imply, that the preparations used and the batyl alcohol should be considered to have a special affinity just for these cells. If the hypothesis is correct as to the lipid membrane the result is, that there will be a wider range of action as indeed is suggested by the results of the experiments on benzene depleted animals. The differences in general condition, tendency to benzene necrosis at the site of the injections and mortality, cannot be adequately explained merely by pointing to the differences in erythrocyte numbers.

Chapter VII

GENERAL SUMMARY

Since 1930 or thereabout there now and then have been published clinical and experimental experiences of therapy of blood disorders, chiefly agranulocytosis, with *per os* or injection preparations made from the nonsaponifiable fat in yellow bone marrow.

The assumption has been, that yellow bone marrow contains a specific leukopoietic principle. The papers published have not afforded any certain support for this assumption, and this has had an impairing effect on the interpretation of the observations made. The possibility has been overlooked that some favourable clinical and experimental findings could be wholly or partly attributed to an antitoxic effect of the used bone marrow preparation.

First the author treated a number of *benzene depleted albino rats* with injection preparations made from nonsaponifiable yellow bone marrow fat from cattle. A number of animals given benzene solely were observed as controls. It turned out, that *the bone marrow therapy increased the number of erythrocytes, decreased the tendency to benzene necrosis at the site of the injection, improved the general condition and lowered the mortality.*

These results could not be explained as being solely due to an improved hematopoiesis, but in the author's opinion they would be chiefly attributable to an antitoxic action of the bone marrow extract. A simultaneous purely hematopoietic action could neither be excluded nor proved by this experiment.

In order to test the hematopoietic action of the preparation a number of *healthy*, i. e. not benzene depleted, *albino rats* were treated. Now the preparation also had been fractionated to a simple compound, which was identified as batyl alcohol (α-n-octadecyl glyceryl ether). It now turned out, that *partly the unfractionated preparation, partly the batyl alcohol and partly the other preparations tested containing relatively large quantities of batyl alcohol increased the number of erythrocytes in the blood.* (If in these experiments the preparations had any effect on the granulocyte count, the author cannot say, as the material is too small to adequately solve this problem.)

The discovery of the effect of batyl alcohol in albino rat caused the author to try *synthetic batyl alcohol* in *healthy man* also. For practical reasons the quantity of substance that could be produced, was very small, and therefore the tested quantity became much smaller in this case than in the animal experiment. The dose given to 10

test persons was sufficient, however, to induce a *significant reticulocytosis* which the author interpreted as being characteristic of a changed bone marrow activity.

In conjunction these two experiments on otherwise untreated test animals and human subjects permit the conclusion, that in normal yellow bone marrow from cattle there is a substance, batyl alcohol, possessing a hematopoietic effect, demonstrated by the experiments at least in respect of the erythropoiesis.

APPENDICES

Appendix No. 1.

The Mean error of M_i.

Individual No.	Observations	Mean	Variance about M_i
1	x_{11} x_{12} x_{13} x_{14} x_{15}	M_1	$\sigma_1^2 = \frac{1}{5}\Sigma x_{1j}^2 - M_1^2$
.	.....	...	.....
i	x_{i1} x_{i2} x_{i3} x_{i4} x_{i5}	M_i	$\sigma_i^2 = \frac{1}{5}\Sigma x_{ij}^2 - M_i^2$
.	.....	...	.....
n	x_{n1} x_{n2} x_{n3} x_{n4} x_{n5}	M_n	$\sigma_n^2 = \frac{1}{5}\Sigma x_{nj}^2 - M_n^2$
	Mean	M	$\sigma_v^2 = \frac{1}{5n}\Sigma\Sigma x_{ij}^2 - \frac{1}{n}\Sigma M_i^2$

Variance for all observations: $\sigma^2 = \frac{1}{5n}\Sigma\Sigma x_{ij}^2 - M^2$

» » M_i-values: $\sigma_M^2 = \frac{1}{n}\Sigma M_i^2 - M^2$

A comparison of the three variances gives:

$$\sigma_v^2 = \sigma^2 - \sigma_M^2 \quad [1]$$

σ_v^2 i however the average variance about the respective *empiric* means. The mathematical expectans for (the mean of) the real variance is:

$$\bar{\sigma}_v^2 = \frac{5}{4}\sigma_v^2$$

$$\text{i. e. } \bar{\sigma}_v^2 = 1.25(\sigma^2 - \sigma_M^2)$$

If this formula is assumed to hold for all individuals the mean error of M_i will be:

$$\varepsilon(M_i) = \frac{\sigma_v}{\sqrt{5}} = \frac{1}{2}\sqrt{\sigma^2 - \sigma_M^2}$$

[1] This formula may be found in for example YULE-KENDALL: An Introduction to the Theory of Statistics 1947 p. 445, formula 23—19.

Appendix No. 2.

Table 15. 100 times the aberration of the observed erythrocyte number from the normal value.

Group F_0.

Animal No.	Before treatment M_i	During treatment									
		Period I			Period II			Period III			Period IV
		20.7	24.7	27.7	31.7	3.8	7.8	10.8	14.8	17.8	21.8	24.8
1	Dead											
2	8.87	—27	61	113	—385	—49	9	—51	—19	—15	—33	dead
3	8.82	—50	—30	—52	—42	—4	—12	—24	20	10	10	10
4	8.24	58	62	Dead								
5	8.00	80	94	126	138	66	68	60	56	88	154	142
6	8.84	50	36	78	—32	20	30	—34	60	70	28	66
7	8.34	24	254	—22	74	64	134	—32	102	74	154	136
8	8.59	55	115	191	57	27	71	—19	167	205	153	157
9	8.45	101	45	—31	—53	—15	67	43	83	75	81	113
10	9.36	38	56	24	Dead							
11	8.69	51	73	39	267	—5	9	—35	51	41	53	—1
12	8.26	52	56	102	64	—20	58	40	134	44	102	84
13	8.15	33	117	111	77	87	83	107	17	17	25	119
14	8.83	9	13	61	67	9	97	17	29	57	63	55
15	8.18	56	52	62	12	74	66	62	82	72	80	64
16	8.15	—17	41	85	39	—85	99	111	59	85	71	69
17	8.44	18	—74	—28	42	42	0	78	58	24	26	62
18	9.32	—32	—28	—26	—16	48	14	32	8	38	—50	22
19	8.33	97	—69	5	71	91	49	—21	69	43	51	103
20	8.86	118	16	42	—28	—40	—68	114	98	24	38	52
Sum		714	890	880	352	310	774	448	1 074	952	1 006	1 253
n	19	19	18	17	17	17	17	17	17	17	17	16
M		0.38	0.47	0.49	0.21	0.18	0.46	0.26	0.63	0.56	0.59	0.78

n = number of animals observed.
M = mean aberration.

Table 16. 100 times the aberration of the observed erythrocyte number from the normal value.

Group F_2.

Animal No.	Before treatment M_i	During treatment									
		Period I			Period II			Period III			Period IV	
		20.7	24.7	27.7	31.7	3.8	7.8	10.8	14.8	17.8	21.8	24.8
21	8.80	—14	—48	—94	72	28	78	—110	12	26	18	64
22	8.47	39	105	—5	95	57	—53	63	59	29	97	75
23	8.39	—17	—15	—39	—17	—61	67	51	71	69	65	109
24	8.57	51	77	—65	71	21	—61	59	133	23	33	3
25	8.50	74	—28	92	50	—36	—26	24	98	42	88	118
26	8.22	86	18	—58	—60	58	82	42	142	126	102	106
27	7.89	49	17	137	145	73	—45	87	185	135	163	257
28	8.47	—45	—11	—59	—45	—83	—95	29	59	25	—17	17
29	7.85	—31	—3	15	—137	17	—31	—21	135	23	—53	143
30	8.41	21	19	17	47	109	51	73	49	125	—25	85
31	7.76	154	49	32	74	120	152	136	54	74	74	136
32	7.74	90	2	—12	—8	116	124	110	160	70	80	132
33	8.07	—37	—185	95	29	—47	101	—31	41	43	117	39
34	8.36	84	48	58	26	—26	40	—44	96	78	—30	84
35	8.92	—20	—126	50	—2	—86	26	—42	—18	8	40	72
36	8.84	—16	116	108	30	88	12	—50	90	78	28	68
37	8.12	36	14	54	104	82	76	—22	126	4	94	82
38	8.34	—36	—10	—6	—60	0	76	—14	74	16	62	22
39	8.22	—14	6	130	72	—68	74	124	58	56	132	34
40	8.71	—131	5	17	21	—41	—117	109	67	—93	5	29
Sum		323	50	479	507	321	531	573	1 691	957	1 073	1 675
n		20	—	—	—	—	—	—	—	—	—	20
M		0.16	0.02	0.24	0.25	0.16	0.27	0.29	0.85	0.48	0.54	0.84

n = number of animals observed.
M = mean aberration.

Table 17. 100 times the aberration of the observed erythrocyte number from the normal value.

Group F_2M.

Animal No.	Before treatment M_i	During treatment										
		Period I			Period II			Period III			Period IV	
		20.7	24.7	27.7	31.7	3.8	7.8	10.8	14.8	17.8	21.8	24.8
41	8.11	—47	49	71	Dead							
42	Dead											
43	8.60	—42	—52	—32	—58	—10	42	—50	—40	42	42	10
44	8.19	31	95	—3	35	17	137	37	149	49	27	—1
45	7.67	—43	27	55	23	—19	—1	21	51	31	41	33
46	8.13	—33	—81	—129	—23	—77	—3	—27	55	1	—3	—7
47	Dead											
48	8.04	24	—32	58	—10	56	—24	28	—46	—92	—120	—60
49	8.12	—4	—14	—60	2	38	—62	—4	18	—34	—70	—12
50	7.42	62	64	—64	52	44	54	148	94	156	36	130
51	7.24	50	98	38	50	94	36	20	144	—42	—96	—10
52	8.30	50	12	6	88	42	2	—38	72	64	—19	132
53	8.02	24	56	—50	72	—68	—30	—6	—30	—20	—54	82
54	7.26	82	18	88	138	40	50	76	140	122	126	122
55	8.35	25	81	—141	—41	—59	—97	—145	—205	—23	79	91
56	8.22	—32	52	—4	—82	—80	—188	—150	—28	—116	—106	—34
57	7.98	—4	—80	—68	4	14	68	34	28	—4	—22	12
58	8.34	—34	—62	—52	—22	—22	62	—16	50	28	—6	—40
59	8.00	42	100	102	70	—6	112	28	94	146	82	68
60	Dead											
Sum		151	331	—185	298	4	158	—44	546	308	—63	516
n	17	17	17	17	16	16	16	16	16	16	16	16
M		0.09	0.19	—0.11	0.18	0.00	0.10	—0.03	0.34	0.19	0.04	0.32

n = number of animals observed.

M = mean aberration.

Table 18. 100 times the aberration of the observed erythrocyte number from the normal value.

Group F_2P.

Animal No.	Before treatment M_i	During treatment										
		Period I			Period II			Period III			Period IV	
		21.7	25.7	28.7	1.8	4.8	8.8	11.8	15.8	18.8	22.8	25.8
61	7.79	71	—41	97	Dead							
62	9.15	251	71	91	87	—111	77	—43	223	—13	Dead	
63	7.64	52	—144	—188	—142	—120	—104	—84	104	100	Dead	
64	7.38	154	102	74	14	62	100	122	178	228	240	122
65	8.23	—21	—25	5	95	81	103	53	85	119	131	43
66	8.14	16	24	40	10	—12	94	2	116	54	46	24
67	8.41	35	23	41	99	35	5	75	139	93	113	31
68	9.50	—178	—98	—100	—64	—254	—44	—48	120	14	—148	—22
69	8.12	—33	—20	—200	100	—18	—42	—38	38	—92	—92	—68
70	9.27	—141	—67	—47	—17	—45	5	—59	13	—33	—39	—1
71	7.72	—10	98	—2	88	100	52	98	80	264	154	132
72	8.54	—46	46	—24	54	—12	100	54	4	74	20	66
73	8.12	64	32	170	138	14	90	74	40	—28	—134	—20
74	7.74	106	—86	—30	82	156	166	Dead				
75	7.72	34	66	26	124	28	88	84	60	62	206	102
76	8.57	17	—201	65	17	75	—17	17	—9	—111	—23	—19
77	8.85	93	—127	35	—9	—19	—41	—55	—75	11	—19	—35
78	8.66	—60	26	34	18	—20	118	—14	—16	126	58	140
79	9.12	—94	—60	—30	—122	14	26	Dead				
80	8.46	34	—42	42	—94	Dead						
Sum		344	—423	99	478	—46	776	238	1 100	868	513	495
n	20	20	20	19	18	18	16	16	16	14	14	14
M		0.17	—0.21	0.05	0.25	—0.03	0.43	0.15	0.69	0.54	0.37	0.35

n = number of animals observed.

M = mean aberration.

Table 19. 100 times the aberration of the observed erythrocyte number from the normal value.

Group F_2A.

Animal No.	Before treatment M_i	During treatment										
		Period I			Period II			Period III			Period IV	
		21.7	25.7	28.7	1.8	4.8	8.8	11.8	15.8	18.8	22.8	25.8
81	8.18	38	50	—6	86	78	14	8	82	—22	22	40
82	7.98	4	—2	46	28	132	—22	74	144	18	—28	—28
83	7.36	—76	84	64	16	152	126	262	64	98	96	68
84	8.21	23	—35	35	25	87	75	113	—89	3	29	—7
85	9.03	—29	1	119	37	95	11	33	17	—17	159	59
86	8.53	7	—71	—71	93	83	—5	65	39	15	—21	47
87	7.79	51	17	—35	51	207	69	29	27	11	61	39
88	8.48	46	—70	—96	—2	22	—42	—4	2	38	—8	6
89	7.88	44	—70	—92	—54	—40	—14	6	66	36	18	40
90	7.78	126	90	Dead								
91	6.92	106	68	118	86	150	64	94	58	110	78	118
92	7.58	84	—26	76	160	40	108	194	146	22	118	52
93	8.14	148	54	12	156	58	72	100	152	84	72	94
94	8.34	60	—48	64	84	4	116	42	198	32	92	30
95	8.72	40	—132	—140	—56	—38	82	32	126	54	44	14
96	8.40	—110	—98	—159	—146	—178	—78	—118	154	—86	—172	—168
97	8.33	—23	—27	31	3	25	7	—131	5	—45	—11	—65
98	8.07	—39	—55	—29	31	65	—35	145	121	17	—3	—1
99	8.29	—53	—73	—27	7	47	67	19	27	—69	—35	—31
100	8.02	26	54	—18	42	—20	62	14	90	—2	—24	84
Sum		473	—289	—108	647	969	677	977	1 429	341	487	391
n	20	20	20	19	—	—	—	—	—	—	—	19
M		0.24	—0.14	—0.06	0.34	0.51	0.36	0.51	0.75	0.18	0.26	0.21

n = number of animals observed.

M = mean aberration.

Table 20. 100 times the aberration of the observed erythrocyte number from the normal value.

Group F_2AK.

Animal No.	Before treatment	During treatment									
		Period I			Period II			Period III			Period IV
	M_i	21.7	25.7	28.7	1.8	4.8	8.8	11.8	15.8	18.8	22.8	25.8
101	8.26	—70	—46	30	—94	18	84	—2	48	—64	40	10
102	8.20	—38	—284	182	28	58	72	—28	102	88	18	26
103	7.51	—35	—27	147	61	99	49	165	307	135	85	83
104	8.37	83	—79	—19	—59	—19	47	97	169	109	23	93
105	8.16	32	—92	32	—4	44	76	78	—10	48	0	30
106	7.97	37	41	117	109	143	—27	95	75	115	111	97
107	9.06	—66	—14	70	64	0	—72	0	64	14	36	132
108	8.10	—18	—30	114	56	—10	26	—122	34	62	120	120
109	8.72	16	10	0	14	58	40	10	22	46	8	8
110	7.58	34	50	76	26	50	72	30	34	100	46	148
111	7.73	1	49	Dead								
112	7.65	15	—3	19	95	75	19	89	59	99	81	43
113	8.45	—75	63	3	—7	23	11	5	1	7	11	25
114	7.78	—32	—194	—68	—20	—52	58	80	98	—14	50	88
115	8.91	29	—1	—61	—177	—151	—111	—61	5	—15	1	57
116	8.04	98	20	88	64	50	—14	4	144	44	120	124
117	8.10	50	66	44	100	110	—24	—64	68	38	34	40
118	7.47	—5	33	59	117	85	59	103	177	107	75	63
119	8.64	—62	—62	62	12	—2	—26	70	118	78	46	120
120	7.55	57	33	115	—65	145	—21	83	115	111	17	83
Sum		51	—467	1 010	320	724	318	632	1 630	1 108	922	1 390
n	20	20	20	19	—	—	—	—	—	—	—	19
M		0.03	—0.23	0.53	0.17	0.38	0.17	0.33	0.86	0.58	0.49	0.73

n = number of animals observed.

M = mean aberration.

Table 21. 100 times the aberration of the observed erythrocyte number from the normal value.

Group F_2K.

Animal No.	Before treatment M_i	During treatment										
		Period I			Period II			Period III			Period IV	
		22.7	26.7	29.7	2.8	5.8	9.8	12.8	16.8	19.8	23.8	26.8
121	8.17	59	69	47	45	91	Dead					
122	7.83	107	—9	33	15	81	—27	—59	—45	—81	Dead	
123	8.24	60	106	—32	38	56	102	66	8	—6	—12	—14
124	7.87	—135	9	—165	—9	103	—107	23	53	47	5	9
125	8.37	—47	—13	—123	—135	—23	33	—17	7	41	—33	1
126	7.14	26	—16	—78	—130	148	78	—42	70	20	104	84
127	7.93	95	—125	—145	—123	13	—13	65	73	81	—25	—91
128	7.67	—67	45	—125	3	67	59	41	65	37	25	13
129	8.02	34	14	—36	52	20	22	—118	116	90	34	—4
130	8.77	11	15	—49	—103	—219	—141	—207	—163	—131	—69	—59
131	8.08	—28	—38	—28	—40	—16	—132	Dead				
132	7.60	0	104	—46	—150	—124	Dead					
133	7.40	—90	106	34	56	14	20	14	60	76	100	52
134	8.82	38	—4	—48	—20	—18	—46	52	—4	—26	—40	—30
135	8.08	76	6	0	—4	36	56	12	32	92	116	68
136	8.65	95	91	—3	13	—5	—47	—21	—11	3	7	—37
137	7.58	96	96	—6	92	134	30	124	104	104	140	132
138	7.01	143	29	47	61	103	65	73	117	129	113	161
139	8.48	98	—66	—122	—64	56	18	—18	70	—30	—16	—2
140	7.88	—32	—54	—46	118	46	38	58	52	—64	79	90
Sum		539	441	—891	—285	563	8	46	604	382	528	373
n	20	—	—	—	—	20	18	17	—	—	16	16
M		0.27	0.22	—0.45	—0.14	0.28	0.00	0.03	0.36	0.22	0.33	0.23

n = number of animals observed.

M = mean aberration.

Table 22. 100 times the aberration of the observed erythrocyte number from the normal value.

Group F_2R.

Animal No.	Before treatment M_i	During treatment										
		Period I			Period II			Period III			Period IV	
		22.7	26.7	29.7	2.8	5.8	9.8	12.8	16.8	19.8	23.8	26.8
141	7.83	39	9	—145	23	83	27	—29	101	61	43	53
142	8.62	—94	—58	—74	—18	—62	—46	—22	50	—40	—10	4
143	7.66	38	36	—90	94	52	36	—4	—108	40	40	42
144	8.03	55	7	13	23	11	—79	—9	1	55	23	31
145	8.86	—12	54	—10	20	20	56	4	50	—42	12	—40
146	7.86	86	48	24	172	76	0	20	96	140	144	112
147	7.91	143	95	28	121	115	25	87	125	75	56	65
148	9.10	—20	36	—20	—122	—4	—28	46	—74	—12	—113	—172
149	8.22	—210	24	—62	24	8	—20	96	80	16	50	6
150	8.15	63	87	—85	17	67	59	47	55	44	15	—27
151	7.85	23	105	39	47	97	61	57	115	131	179	77
152	7.76	—52	—12	—78	30	8	—4	—62	58	32	74	100
153	8.36	48	68	—46	44	22	—2	—10	66	32	4	16
154	8.32	—130	32	—76	22	—10	8	128	74	—8	—50	0
155	8.91	—25	41	—97	—99	—113	—23	7	—231	—1	—29	27
156	8.10	—12	2	22	38	22	46	58	—8	—38	42	14
157	8.02	2	—4	—108	—32	—228	—212	—20	—130	—174	—188	—126
158	7.54	12	—92	24	18	—18	—116	46	74	—94	—184	—144
159	8.53	—1	—67	—55	—173	—265	—281	—139	—263	—199	—309	—333
160	7.53	101	37	61	71	165	15	—185	—83	—133	—91	—101
Sum		54	448	—735	320	46	—478	116	48	—115	—292	—396
n	20	—	—	—	—	—	—	—	—	—	—	20
M		0.03	0.22	—0.37	0.16	0.02	—0.24	0.06	0.02	—0.06	—0.15	—0.20

n = number of animals observed.

M = mean aberration.

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